THE UNITED STATES PATENT AND TRADEMARK OFFICE
BEFORE THE BOARD OF PATENT APPEALS AND INTERFERENCES

Appellant:	Malnoe, et al.
Appl. No.:	10/607,330
Conf. No.:	4205
Filed:	June 26, 2003
Title:	COMPOSITIONS AND METHODS AGAINST INFLAMMATORY PROCESSES
Art Unit:	1655
Examiner:	Deborah A. Davis
Docket No.:	115808-365

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

APPELLANTS' APPEAL BRIEF

Sir:

Appellants submit this Appeal Brief in support of the Notice of Appeal filed on February 13, 2009. This Appeal is taken from the Final Rejection dated August 15, 2008.

I. REAL PARTY IN INTEREST

The real party in interest for the above-identified patent application on Appeal is Nestec S.A. by virtue of an Assignment recorded August 16, 2004 at reel 015678, frames 0164 to 0170 in the United States Patent and Trademark Office.

II. RELATED APPEALS AND INTERFERENCES

Appellants' legal representative and the Assignee of the above-identified patent application do not know of any prior or pending appeals, interferences of judicial proceedings which may be related to, directly affect or be directly affected by or have a bearing on the Board's decision with respect to the above-identified Appeal.

III. STATUS OF CLAIMS

Claims 1, 3, 4, 6, 8, 10, 11, 14, 16 and 23-64 are pending in the above-identified patent application. Claims 2, 5, 7, 9, 12-13, 15 and 17-22 were previously rejected. Claims 23-62 were previously withdrawn. Claims 1, 3, 4, 6, 8, 10, 11, 14, 16, 63 and 64 stand rejected. Though Claim 14 stands rejected, the Examiner has not specifically addressed Claim 14 in any of the current rejections. Therefore, Claims 1, 3, 4, 6, 8, 10, 11, 14, 16, 63 and 64 are being appealed in this Brief. A copy of the appealed claims is included in the Claims Appendix.

IV. STATUS OF AMENDMENTS

A final Office Action was mailed on August 15, 2008. Appellants filed a Response on November 12, 2008 in reply to the final Office Action. An Advisory Action was mailed on February 5, 2009. A copy of the final Office Action, the Advisory Action and the Response are attached as Exhibits A, B and C, respectively, in the Evidence Appendix.

V. SUMMARY OF CLAIMED SUBJECT MATTER

A summary of the invention by way of reference to the specification and/or figures for each of the independent claims is provided as follows:

Independent Claim 1 is directed to a composition comprising a therapeutically effective amount of a thermally extruded plant material (page 4, lines 18-19) that includes one or more phytochemical agents capable of inhibiting at least one of enzymatic and transcriptional activity to inhibit inflammation in a mammal (page 4, lines 19-22; and page 9, lines 12-14), wherein the one or more phytochemical agents is selected from the group consisting of sesquiterpene lactones, prebiotic fibers, dietary agents, and combinations thereof (page 8, lines 24-32, page 9, lines 4-11 and 23-26; and page 11, lines 8-11), and wherein the plant material comprises an amount from at least 0.5% to less than 5% (page 3, lines 24-25; page 16, lines 7-9 and 28-29; and page 17, lines 20-21) by weight of the composition and wherein the composition further comprises a component selected from the group consisting of a starch source, a protein source, a fat source and combinations thereof (page 11, lines 17-28).

Independent Claim 11 is directed to a composition comprising a therapeutically effective amount of a thermally extruded plant material (page 4, lines 18-19) that includes one or more phytochemical agents capable of inhibiting at least one of enzymatic and transcriptional activity to treat inflammation in a mammal (page 4, lines 19-22; and page 9, lines 12-14) wherein the phytochemical agents include an effective amount of sesquiterpene lactones including an active fragment that includes α-methylene-γ-butyrolactone (page 8, lines 24-32) and wherein the plant material comprises an amount from at least 0.5% to less than 5% by weight of the composition (page 3, lines 24-25; page 16, lines 7-9 and 28-29; and page 17, lines 20-21) and wherein the composition further comprises a component selected from the group consisting of a starch source, a protein source, a fat source and combinations thereof (page 11, lines 17-28).

Independent Claim 63 is directed to a composition comprising an active fragment derived from a thermally extruded plant material (page 4, lines 18-19), the active fragment including α-methylene-γ-butyrolactone wherein the active fragment in an effective amount is capable of inhibiting at least one of enzyme and transcriptional activity to inhibit inflammation (page 4, lines 19-22; and page 9, lines 12-14), wherein the plant material comprises an amount from at least 0.5% to less than 5% by weight of the composition (page 3, lines 24-25; page 16, lines 7-9

and 28-29; and page 17, lines 20-21) and wherein the composition further comprises a component selected from the group consisting of a starch source, a protein source, a fat source and combinations thereof (page 11, lines 17-28).

Although specification citations are given in accordance with C.F.R. 1.192(c), these reference numerals and citations are merely examples of where support may be found in the specification for the terms used in this section of the Brief. There is no intention to suggest in any way that the terms of the claims are limited to the examples in the specification. As demonstrated by the references numerals and citations below, the claims are fully supported by the specification as required by law. However, it is improper under the law to read limitations from the specification into the claims. Pointing out specification support for the claim terminology as is done here to comply with rule 1.192(c) does not in any way limit the scope of the claims to those examples from which they find support. Nor does this exercise provide a mechanism for circumventing the law precluding reading limitations into the claims from the specification. In short, the references numerals and specification citations are not to be construed as claim limitations or in any way used to limit the scope of the claims.

VI. GROUNDS OF REJECTION TO BE REVIEWED ON APPEAL

1. Claims 1, 4, 6, 8 and 10 are rejected under 35 U.S.C. §102(b) as being anticipated by U.S. Patent No. 6,197,361 to Anantharaman et al ("*Anantharaman*"). A copy of *Anantharaman* is attached herewith as Exhibit D.
2. Claims 3, 11, 16, 63 and 64 are rejected under 35 U.S.C. 103(a) as being unpatentable over *Anantharaman* in view of U.S. Patent No. 5,905,089 to Hwang et al. ("*Hwang*"). A copy of *Hwang* is attached herewith as Exhibit E.

VII. ARGUMENT

A. LEGAL STANDARDS

1. Anticipation under 35 U.S.C. § 102

Anticipation is a factual determination that "...requires the presence in a single prior art disclosure of each and every element of a claimed invention." *Lewmar Marine, Inc. v. Barient, Inc.*, 3 U.S.P.Q. 2d 1766 (Fed. Cir. 1987). Moreover, "[a] claim is anticipated only if each and every element as set forth in the claim is found, either expressly or inherently described, in a *single* prior art reference." *Verdegaal Bros. v. Union Oil of California*, 2 U.S.P.Q.2d 1051, 1053 (Fed. Cir. 1987) (*emphasis added*).

Federal Circuit decisions have repeatedly emphasized the notion that anticipation cannot be found where less than all elements of a claimed invention are set forth in a reference. *See, e.g. Transclean Corp. v. Bridgewood Services, Inc.*, 290 F.3d 1364 (Fed. Cir. 2002). In this regard, a reference disclosing "substantially the same thing" is not enough to anticipate. *Jamesbury Corp. v. Litton Indust. Prod., Inc.*, 756 F.2d 1556, 1560 (Fed. Cir. 1985). A reference must clearly disclose each and every limitation of the claimed invention before anticipation may be found.

Further, anticipation cannot be shown by combining more than one reference to show the elements of the claimed invention. *In re Saunders*, 444 F.2d 599 (C.C.P.A. 1971). All elements of a claimed invention must be disclosed in one, solitary reference. As such, it is clear that a reference cannot be utilized to render a claimed invention anticipated without identical disclosure.

2. Obviousness under 35 U.S.C. § 103

The Federal Circuit has held that the legal determination of an obviousness rejection under 35 U.S.C. § 103 is:

> whether the claimed invention as a whole would have been obvious to a person of ordinary skill in the art at the time the invention was made...The foundational facts for the prima facie case of obviousness are: (1) the scope and content of the prior art; (2) the difference between the prior art and the claimed invention; and (3) the level of ordinary skill in the art...Moreover, objective indicia such as commercial success and long felt need are relevant

> to the determination of obviousness...Thus, each obviousness determination rests on its own facts.

In re Mayne, 41 U.S.P.Q. 2d 1451, 1453 (Fed. Cir. 1997).

In making this determination, the Patent Office has the initial burden of proving a *prima facie* case of obviousness. *In re Rijckaert*, 9 F.3d 1531, 1532, 28 U.S.P.Q. 2d 1955, 1956 (Fed. Cir. 1993). This burden may only be overcome "by showing some objective teaching in the prior art or that knowledge generally available to one of ordinary skill in the art would lead that individual to combine the relevant teachings." *In re Fine*, 837 F.2d 1071, 1074, 5 U.S.P.Q. 2d 1596, 1598 (Fed. Cir. 1988). "If the examination at the initial stage does not produce a prima facie case of unpatentability, then without more the applicant is entitled to grant of the patent." *In re Oetiker*, 24 U.S.P.Q. 2d 1443, 1444 (Fed. Cir. 1992).

Of course, references must be considered as a whole and those portions teaching against or away from the claimed invention must be considered. *Bausch & Lomb, Inc. v. Barnes-Hind/Hydrocurve Inc.*, 796 F.2d 443 (Fed. Cir. 1986). "A prior art reference may be considered to teach away when a person of ordinary skill, upon reading the reference would be discouraged from following the path set out in the reference, or would be led in a direction divergent from the path that was taken by the Applicant." *Monarch Knitting Machinery Corp. v. Fukuhara Industrial Trading Co., Ltd.*, 139 F.3d 1009 (Fed. Cir. 1998), quoting, *In re Gurley*, 27 F.3d 551 (Fed. Cir. 1994).

B. THE CLAIMED INVENTION

Independent Claim 1 recites, in part, a thermally extruded plant material that includes one or more phytochemical agents capable of inhibiting at least one of enzymatic and transcriptional activity to inhibit inflammation in a mammal, wherein the one or more phytochemical agents is selected from the group consisting of sesquiterpene lactones, prebiotic fibers, dietary agents, and combinations thereof. Independent Claim 11 recites, in part, a thermally extruded plant material that includes one or more phytochemical agents capable of inhibiting at least one of enzymatic and transcriptional activity to treat inflammation in a mammal. Independent Claim 63 recites, in part, a thermally extruded plant material, the active fragment including α-methylene-γ-

butyrolactone, wherein the active fragment in an effective amount is capable of inhibiting at least one of enzyme and transcriptional activity to inhibit inflammation.

Appellants have surprisingly discovered that upon thermal extruding, certain plants and/or plant extracts thereof can be generated with enhanced inhibition of enzyme activity and/or transcription activity in mammals that is believed to reduce the risk of inflammation. Structurally, therefore, the present claims require, in part, a plant material thermally extruded to inhibit at least one of enzymatic or transcriptional activity to treat inflammation. These are significant structural advantages, considering that the inflammation inhibiting nature of thermally extruded plant material of the present claims is a unique aspect of the invention. Teachings and examples in the specification supporting and elucidating the scope of the present invention include page 3, lines 24-25; page 4, lines 18-22; page 8, lines 24-32; page 9, lines 4-14 and 23-26; page 11, lines 8-11 and 17-28; page 16, lines 7-9 and 28-29; and page 17, lines 17-20.

C. THE REJECTION OF CLAIMS 1, 4, 6, 8 AND 10 UNDER 35 U.S.C. §102(B) SHOULD BE REVERSED BECAUSE THE CITED REFERENCE DOES NOT ANTICIPATE THE CLAIMED INVENTION

The Examiner alleges that *Anantharaman* discloses every element of the Claims 1, 4, 6, 8 and 10. Independent Claim 1 recites, in relevant part, a thermally extruded plant material that includes one or more phytochemical agents capable of inhibiting at least one of enzymatic and transcriptional activity to inhibit inflammation in a mammal, wherein the one or more phytochemical agents is selected from the group consisting of sesquiterpene lactones, prebiotic fibers, dietary agents, and combinations thereof. In contrast, Appellants respectfully submit that the *Anantharaman* fails to disclose or suggest every element of Claims 1, 4, 6, 8 and 10.

For example, Appellants respectfully submit that *Anantharaman* fails to disclose or suggest a composition comprising one or more phytochemical agents capable of inhibiting at least one of enzymatic and transcriptional activity to inhibit inflammation in a mammal, wherein the one or more phytochemical agents is selected from the group consisting of sesquiterpene lactones, prebiotic fibers, dietary agents, and combinations thereof. Appellants respectfully disagree with the Examiner's assertion that *Anantharaman* discloses that chicory comprises sesquiterpene lactones in a concentration of at least 0.5% by weight. See, final Office Action,

page 3, lines 4-6. The Examiner cites column 1, lines 62-67; column 2, lines 21-65 and Claims 1-7 of *Anantharaman* to support this statement. However, *Anantharaman* specifically teaches the need to destroy or remove these sesquiterpene lactones. See, *Anantharaman,* column 6, lines 30-34. Furthermore, contrary to the Examiner's assertion, the sections of *Anantharaman* cited by the Examiner do not disclose that the sesquiterpene lactones are still present. In fact, the final mixture is analyzed and "[n]o sesquiterpene lactones are detected". See, *Anantharaman,* column 7, lines 29-31. The sesquiterpene lactones described were only in the chicory starting ingredient and not in the final mixture administered to the dogs. See, *Anantharaman,* column 7, lines 31-34. Therefore, *Anantharaman* fails to disclose sesquiterpene lactones present in a composition such that they can inhibit inflammation in mammal as required by independent Claim 1.

In response, the Examiner asserts that *Anantharaman* teaches the addition of santonin to the chicory mixture, with santonin being a type of sesquiterpene lactone. See, Advisory Action, page 2. Regardless of the accuracy of the Examiner's assertion, it still does not change subsequent disclosure in *Anantharaman*, which teaches, "the matrix making up the cereal product must be gelatinized in order to destroy or remove the sesquiterpene compounds present in the inulin-containing material" (emphasis added). See, *Anantharaman,* column 6, lines 30-34. Therefore, even if sesquiterpene lactones are added to a mixture, subsequent processing must occur to remove completely the sesquiterpene lactones as described above. Consequently, no sesquiterpene lactones can be present when administered to a mammal to inhibit inflammation in the mammal as required by the claims.

The Examiner further asserts that because the final mixture was analyzed by HPLC for bound sesquiterpene lactones (i.e., not free lactones) and none was found, *Anantharaman* still anticipates the instant claims because sesquiterpene lactones are found in the composition of chicory. See, Advisory Action, page 2. Appellants strongly disagree with this assertion. First, the assertion is incorrect, for *Anantharaman* states as follows:

> The pellets are crushed and extracted with methanol by boiling under reflux for 1 hour. The extract is twice partitioned between water and chloroform and santonin is added. The chloroform phase is separated, dried and evaporated. The residue is dissolved in a mixture of methanol and chloroform and analyzed using HPLC for ***free*** sesquiterpene lactones. The water phase is run through a column and glycosylated compounds eluted from the column using methanol. The eluant is evaporated, dissolved in water and treated with cellulase at 40.degree. C. for 2 hours. Santonin is added to the hydrolysate and the mixture extracted with ethyl

> acetate. The mixture is then analyzed using HPLC for ***bound*** sesquiterpene lactones. No sesquiterpene lactones are detected.

See, *Anantharaman*, column 7, lines 18-33. As is clear from the above passage, HPLC testing is done for both free and bound sesquiterpene lactones, and "[n]o sesquiterpene lactones are detected." Second, even if the HPLC procedure conducted in *Anantharaman* was somehow limited to bound sesquiterpene lactones, which Appellants argue above as incorrect, this does mean that free lactones are somehow still present. As discussed previously, *Anantharaman* states that processing occurs to remove or destroy sesquiterpene compounds. *Anantharaman* speaks plainly and does not limit the type of sesquiterpene compounds removed or destroyed. Therefore, regardless of the interpretation of the HPLC testing procedure, *Anantharaman* clearly does not teach or suggest the presence of sesquiterpene lactones in a composition when administered to a mammal such that the composition is able to inhibit at least one of enzyme and transcriptional activity to inhibit inflammation.

As such, Appellants submit that *Anantharaman* further fails to disclose or suggest a thermally extruded plant material that includes one or more phytochemical agents capable of inhibiting at least one of enzymatic and transcriptional activity to inhibit inflammation in a mammal. As discussed above, *Anantharaman* fails to disclose or suggest sesquiterpene lactones present in the extract capable of inhibiting at least one of enzymatic and transcriptional activity to inhibit inflammation in a mammal. Therefore, *Anantharaman* must also fail to disclose a thermally extruded plant material having one or more phytochemical agents in order to inhibit inflammation.

As discussed previously, *Anantharaman* discloses a composition without any sesquiterpene lactones left in the composition to inhibit cyclooxygenase activity to inhibit inflammation in a mammal. These are significant structural differences, considering that the inflammation inhibiting nature of the present claims is a unique aspect of the invention and is not disclosed or suggested by *Anantharaman*.

The Examiner asserts, however, that the inhibition of enzymatic and transcriptional activity would be inherent because both the claims and *Anantharaman* teach thermally extruded chicory plant material. See, Advisory Action, page 2. For the reasons just discussed previously, Appellants submit that the claims are distinguishable because the composition in *Anantharaman* is processed specifically to remove sesquiterpene lactones, which is one agent capable of

inhibiting the enzymatic and transcriptional activity in a mammal as required by the claims. Because that component is processed out of the composition, the composition in *Anantharaman* lacks the required elements, and is therefore deficient with respect to the present claims.

For the reasons discussed above, Appellants respectfully submit that Claim 1 and Claims 4, 6, 8 and 10 that depend from Claim 1 are novel, non-obvious and distinguishable over *Anantharaman*. Accordingly, Appellants respectfully request that the rejection of Claims 1, 4, 6, 8 and 10 under 35 U.S.C. §102(b) be withdrawn.

D. THE REJECTION OF CLAIMS 3, 11, 16, 63 AND 64 UNDER 35 U.S.C. §103(A) SHOULD BE REVERSED BECAUSE THE CITED REFERENCES DO NOT RENDER OBVIOUS THE CLAIMED INVENTION

The Examiner alleges that the cited references, in combination, disclose every element of the Claims 3, 11, 16, 63 and 64. Independent Claim 1 requires, in part, a thermally extruded plant material that includes one or more phytochemical agents capable of inhibiting at least one of enzymatic and transcriptional activity to inhibit inflammation in a mammal, wherein the one or more phytochemical agents is selected from the group consisting of sesquiterpene lactones, prebiotic fibers, dietary agents, and combinations thereof. Similarly, independent Claim 11 requires, in part, a thermally extruded plant material that includes one or more phytochemical agents capable of inhibiting at least one of enzymatic and transcriptional activity to treat inflammation in a mammal. Furthermore, independent Claim 63 requires, in part, a thermally extruded plant material, the active fragment including α-methylene-γ-butyrolactone, wherein the active fragment in an effective amount is capable of inhibiting at least one of enzyme and transcriptional activity to inhibit inflammation. In contrast, Appellants respectfully submit that there exists no reason why the skilled artisan would combine the cited references to arrive at Claims 3, 11, 16, 63 and 64.

For example, as previously discussed, *Anantharaman* discloses destroying or removing sesquiterpene lactones. Specifically, *Anantharaman* teaches the need to "destroy or remove" these sesquiterpene lactones. See, *Anantharaman,* col. 6, lines 30-34. In contrast, *Hwang* is entirely directed toward using sesquiterpene lactones obtained from plant material. See, *Hwang*, Abstract. As stated above in response to the anticipation rejection and in response to the

Examiner's assertions, *Anantharaman* includes processing steps designed specifically to remove or destroy sesquiterpene lactones. Example 1 in *Anantharaman*, as also discussed above, tests for both free and bound sesquiterpene lactones, with the tests ultimately revealing that no sesquiterpene lactones are detected. This meets the requirements set forth in *Anantharaman* and, therefore, specifically teaches away from the use of sesquiterpene lactones arguably taught in *Hwang*.

Further, references are not properly combinable or modifiable if their intended purpose is destroyed. For instance, if the proposed modification would render the prior art invention being modified unsatisfactory for its intended purpose, then there is no suggestion or motivation to make the proposed modification. *In re Gordon*, 733 F.2d 900, 221 USPQ 1125 (Fed. Cir. 1984). This is exactly the case where *Anantharaman* is directed toward a matrix making up a cereal product that must be gelatinized in order to "remove or destroy the sesquiterpene compounds" present in the plant material (see, *Anantharaman,* col. 6, lines 30-34), and where *Hwang* is entirely directed toward using sesquiterpene lactones obtained from plant material (see, *Hwang*, Abstract).

Appellants respectfully submit that the claims must be viewed as a whole as defined by the claimed invention and not dissected into discrete elements to be analyzed in isolation. *W.L. Gore & Assoc., Inc. v. Garlock, Inc.*, 721 F.2d 1540, 1548, 220 USPQ 303, 309 (Fed. Cir. 1983); *In re Ochiai*, 71 F.3d 1565, 1572, 37 USPQ2d 1127, 1133 (Fed. Cir. 1995). Further, one should not use hindsight reconstruction to pick and choose among isolated disclosures in the prior art to deprecate the claimed invention. *In re Fine*, 837 F.2d at 1075. (Fed. Cir. 1988).

Even if combinable, the cited references, in combination, are still deficient. *Anantharaman* teaches the removal or destruction of sesquiterpene lactones through specific processing steps for that purpose. Therefore, even if *Hwang* teaches the addition of sesquiterpene lactones to a composition, the processing steps detailed in *Anantharaman* would still remove any added sesquiterpene lactones, as is the objective in *Anantharaman*.

For at least the reasons discussed above, the skilled artisan would have no reason to combine *Anantharaman* and *Hwang* to arrive at the present claims. Appellants respectfully submit therefore that the combination of *Anantharaman* and *Hwang* is improper. Moreover, even if combinable, Appellants submit that the cited references, in combination, are still deficient.

Accordingly, Appellants respectfully request that the obviousness rejection with respect to Claims 3, 11, 16 and 63-64 be reconsidered and withdrawn.

E. CONCLUSION

Appellants respectfully submit that the Examiner has failed to establish anticipation under 35 U.S.C. §102 with respect to the rejection of Claims 1, 4, 6, 8 and 10 and a *prima facie* case of obviousness under 35 U.S.C. §103 with respect to the rejection of Claims 3, 11, 16, 63 and 64. Accordingly, Appellants respectfully submit that the anticipation and obviousness rejections are erroneous in law and in fact and should therefore be reversed by this Board.

The Director is authorized to charge any fees that may be required, or to credit any overpayment to Deposit Account No. 02-1818. If such a withdrawal is made, please indicate the Attorney Docket No. 115808-365 on the account statement.

Respectfully submitted,

K&L GATES LLP

BY _____

Robert M. Barrett
Reg. No. 30,142
Customer No. 29157
Phone No. 312.807.4204

Dated: May 8, 2009

CLAIMS APPENDIX

PENDING CLAIMS ON APPEAL OF U.S. PATENT APPLICATION SERIAL NO. 10/607,330

1. A composition comprising a therapeutically effective amount of a thermally extruded plant material that includes one or more phytochemical agents capable of inhibiting at least one of enzymatic and transcriptional activity to inhibit inflammation in a mammal, wherein the one or more phytochemical agents is selected from the group consisting of sesquiterpene lactones, prebiotic fibers, dietary agents, and combinations thereof, and wherein the plant material comprises an amount from at least 0.5% to less than 5% by weight of the composition and wherein the composition further comprises a component selected from the group consisting of a starch source, a protein source, a fat source and combinations thereof.

3. The composition according to claim 1, wherein the plant material contains an effective amount of sesquiterpene lactones including an active fragment thereof that includes α-methylene-γ-butyrolactone.

4. The composition according to claim 1, wherein the plant material is selected from the group consisting of chicory, lettuce, coffee, soja, Jerusalem artichoke, leek, onion, yacon, asparagus, extracts thereof and combinations thereof.

6. The composition according to claim 1, wherein the plant material comprises a chicory extract.

8. The composition according to claim 1, wherein one or more of the phytochemical agents are capable of inhibiting at least one of enzymatic activity derived from cyclooxygenase and transcriptional activity derived from NF-κB.

10. The composition according to claim 1, wherein the plant material that is thermally processed includes an extruded plant material.

11. A composition comprising a therapeutically effective amount of a thermally extruded plant material that includes one or more phytochemical agents capable of inhibiting at least one of enzymatic and transcriptional activity to treat inflammation in a mammal wherein the phytochemical agents include an effective amount of sesquiterpene lactones including an active fragment that includes α-methylene-γ-butyrolactone and wherein the plant material comprises an amount from at least 0.5% to less than 5% by weight of the composition and wherein the composition further comprises a component selected from the group consisting of a starch source, a protein source, a fat source and combinations thereof.

14. The composition according to claim 11, wherein the plant material comprises a chicory extract.

16. The composition according to claim 11, wherein one or more of the phytochemical agents are capable of inhibiting at least one of enzymatic activity derived from cyclooxygenase and transcriptional activity derived from NF-κB.

63. A composition comprising an active fragment derived from a thermally extruded plant material, the active fragment including α-methylene-γ-butyrolactone wherein the active fragment in an effective amount is capable of inhibiting at least one of enzyme and transcriptional activity to inhibit inflammation, wherein the plant material comprises an amount from at least 0.5% to less than 5% by weight of the composition and wherein the composition further comprises a component selected from the group consisting of a starch source, a protein source, a fat source and combinations thereof.

64. The composition according to claim 63, wherein the active fragment is capable of inhibiting at least one of enzymatic activity derived from cyclooxygenase and transcriptional activity derived from NF-κB.

EVIDENCE APPENDIX

EXHIBIT A: Final Office Action mailed August 15, 2008.

EXHIBIT B: Advisory Action mailed February 5, 2009.

EXHIBIT C: November 12, 2008 Response to final Office Action mailed August 15, 2008.

EXHIBIT D: U.S. Patent No. 6,197,361 to Anantharaman et al. ("*Anantharaman*").

EXHIBIT E: U.S. Patent No. 5,905,089 to Hwang et al. ("*Hwang*").

RELATED PROCEEDINGS APPENDIX

None

Exhibit A

EXHIBIT A

UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
P.O. Box 1450
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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
10/607,330	06/26/2003	Armand Malnoe	115808-365	4205

29157 7590 08/15/2008

BELL, BOYD & LLOYD LLP
P.O. Box 1135
CHICAGO, IL 60690

EXAMINER
DAVIS, DEBORAH A

ART UNIT	PAPER NUMBER
1655	

NOTIFICATION DATE	DELIVERY MODE
08/15/2008	ELECTRONIC

Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Notice of the Office communication was sent electronically on above-indicated "Notification Date" to the following e-mail address(es):

PATENTS@BELLBOYD.COM

EXHIBIT A

Office Action Summary	Application No.	Applicant(s)	
	10/607,330	MALNOE ET AL.	
	Examiner	Art Unit	
	DEBORAH A. DAVIS	1655	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *05 February 2008*.

2a)☒ This action is **FINAL**. 2b)☐ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1,3,4,6,8,10,11,14,16 and 23-64* is/are pending in the application.

4a) Of the above claim(s) *23-62* is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☐ Claim(s) *1,3,4,6,8,10,11,14,16,63 and 64* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☐ Information Disclosure Statement(s) (PTO/SB/08) Paper No(s)/Mail Date _____.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____.
5) ☐ Notice of Informal Patent Application
6) ☐ Other: _____.

DETAILED ACTION

Applicants' response to the Office Action mailed on February 5, 2008 is acknowledged. Currently, claims 1, 3-4, 6, 8, 10-11, 14,16, and 23-64 are pending, claims 23-62 are withdrawn, claims 1, 11 and 63 are amended. Claim 2, 5, 7, 9, 12-13, 15, and 17-22 are cancelled. Claims 1, 3-4, 6, 8, 10-11, 14, 16, 63, and 64 are under consideration for examination.

Claim Rejections - 35 USC § 102

The following is a quotation of the appropriate paragraphs of 35 U.S.C. 102 that form the basis for the rejections under this section made in this Office action:

> A person shall be entitled to a patent unless –
>
> (b) the invention was patented or described in a printed publication in this or a foreign country or in public use or on sale in this country, more than one year prior to the date of application for patent in the United States.

Claims 1, 4, 6, 8, and 10 are rejected under 35 U.S.C. 102(b) as being anticipated by Anantharaman et al (US 6,197,361).

A composition comprising a therapeutically effective amount of a thermally extruded plant material that includes one or more phytochemical agents capable of inhibiting at least one of enzymatic and transcriptional activity to inhibit inflammation in a mammal, wherein the one or more phytochemical agents are selected from the group consisting of sesquiterpene lactones, prebiotic fibers, dietary agents, and combinations thereof, and wherein the plant material comprises at least 0.5% to less than 5% by weight of the composition and wherein the composition further comprising a component selected from the group consisting of a starch source, a protein source, a fat source and combinations thereof is apparently claimed.

The reference of Anantharaman et al., anticipates the instant claims by disclosing a gelantinized cereal product comprising chicory plant material and a protein source. The chicory plant material is an extrusion cooked (i.e. thermal) product and may be in a dried pellet form (i.e. fraction). Anantharaman discloses that chicory comprise of sesquiterpene lactones in a concentration of at least 0.5% by weight (column 1, lines 62-67, column 2, lines 21-65, claims 1-7, e.g.). Please note that because there is not a difference in the cited composition and the instant claims, the functional effects of inhibiting at least one of the enzymatic and transcriptional activity, inflammation in a mammal would be inherent.

Therefore, the reference is deemed to anticipate the instant claims.

Claim Rejections - 35 USC § 103

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

Claims 3, 11, 16, 63 and 64 are rejected under 35 U.S.C. 103(a) as being unpatentable over Anantharaman et al as applied to claims 1, 4, 6, 8 and 10 above, and further in view of Hwang et al (US 5,905.089).

The teaching of Anantharaman et al has been set forth above but is silent with respect to particular lactones which include α-methylenebutyrolactone.

The reference of Hwang et al beneficially teaches therapeutically effective amounts of sesquiterpene lactones from plant extracts (composition) which includes α-methylenebutyrolactones that is capable of inhibiting or reducing the severity of a severe inflammatory response from enzyme activity such as cyclooxygenase and transcriptional activity derived from NF-kB as instantly claimed. The composition further includes a fat source such as olive oil, as claimed. Such active sesquiterpene lactones may be used in various combination or mixtures (see abstract, column 5, lines 1-5, lines 40-65, column 5, lines 1-9, column 6, lines 14-36, Examples 1, 4 and 5, e.g.).

It would have been obvious to one of ordinary skill in the art at the time the invention was made to further include into the composition of Anantharaman the α-methylenebutyrolactones taught by Hwang based on the beneficial teachings of treating severe inflammatory disorders. The adjustment of particular conventional working conditions is deemed merely a matter of judicious selection and routine optimization, which is well within the purview of the skilled artisan.

From the teachings of the references, it is apparent that one of ordinary skill in the art would have had a reasonable expectation of success in producing the claimed invention. Therefore, the invention as a whole was prima facie obvious to one of ordinary skill in the art at the time the invention was made, as evidenced by the references, especially in the absence of the evidence to the contrary.

Response to Arguments

Applicant argues that the skilled artisan would have no reason to combine the cited references to obtain the present claims because the cited references teach away from each other and the claimed invention. Applicant argues that the reference of Ernest is directed toward topical and oral herbal composition that can be used to enhance breasts in human females by strengthening connective tissues and encouraging the growth of new cells while Hwang discloses sesquiterpene lactones obtained from plant material. Applicant argues that the reference of Hermand discloses extraction at low temperatures because low temperatures allow good preservation of the resulting extract without the addition of preservative. Applicant further argues that Hermand avoids thermal degradation of the chicory which might possibly denature the active compounds. Applicant concludes that one skilled in the art would have no reason to thermally extrude the plant material of Hermand or to combine Hermand with the cited references of Ernest and Hwang to obtain the present claims. Applicant further concludes that Hermand's hot extracted chicory extract has the same properties as cold extracted chicory and therefore teaches away from the claimed invention. Applicant finally concludes that they have obtained enhanced inhibition of enzyme activity and/or transcription activity in mammals by thermally extruding the plant material which is now required by the instant claims. These arguments has been carefully considered but not found to be persuasive of error.

In response, the cited reference of Ernest teaches plant material that includes the sesquiterpene lactones, which is a phytochemical extracted from Blessed thistle. The plant materials are thermally processed and has evidenced of anti-inflammatory properties. With respect to the reference of Hermand, the examiner only relied on this reference for the teaching of chicory plant material. Applicants' argument with respect to teaching away from the claimed invention is not persuasive because the instant claims do not recite specific temperatures but only recited thermal processing - which is now amended to "thermal extruded". The reference of Hermand used temperatures ranges of 170 degrees or less to process the chicory plant material and therefore met the previous claimed limitation of "thermally processing".

Applicant has now amended the claims and argues that the cited references of Ernest, Hwang and Hermand all fail to disclose or suggest the amended subject matter of thermally extruding a plant material that is now required by independent claims 1, 11 and 63. The above arguments have been carefully considered but are moot in view of the new ground(s) of rejection above.

Conclusion

No claims are allowed.

Applicant's amendment necessitated the new ground(s) of rejection presented in this Office action. Accordingly, **THIS ACTION IS MADE FINAL.** See MPEP § 706.07(a). Applicant is reminded of the extension of time policy as set forth in 37 CFR 1.136(a).

A shortened statutory period for reply to this final action is set to expire THREE MONTHS from the mailing date of this action. In the event a first reply is filed within TWO MONTHS of the mailing date of this final action and the advisory action is not mailed until after the end of the THREE-MONTH shortened statutory period, then the shortened statutory period will expire on the date the advisory action is mailed, and any extension fee pursuant to 37 CFR 1.136(a) will be calculated from the mailing date of the advisory action. In no event, however, will the statutory period for reply expire later than SIX MONTHS from the date of this final action.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to DEBORAH A. DAVIS whose telephone number is (571)272-0818. The examiner can normally be reached on 8-5 Monday thru Friday.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Terry McKelvey can be reached on (571) 272-0775. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

Deborah A. Davis
Patent Examiner, AU 1655
August 2008

/Christopher R. Tate/
Primary Examiner, Art Unit 1655

EXHIBIT A

Notice of References Cited	Application/Control No. 10/607,330	Applicant(s)/Patent Under Reexamination MALNOE ET AL.	
	Examiner DEBORAH A. DAVIS	Art Unit 1655	Page 1 of 1

U.S. PATENT DOCUMENTS

*		Document Number Country Code-Number-Kind Code	Date MM-YYYY	Name	Classification
*	A	US-6,197,361	03-2001	Anantharaman et al.	426/560
	B	US-			
	C	US-			
	D	US-			
	E	US-			
	F	US-			
	G	US-			
	H	US-			
	I	US-			
	J	US-			
	K	US-			
	L	US-			
	M	US-			

FOREIGN PATENT DOCUMENTS

*		Document Number Country Code-Number-Kind Code	Date MM-YYYY	Country	Name	Classification
	N					
	O					
	P					
	Q					
	R					
	S					
	T					

NON-PATENT DOCUMENTS

*		Include as applicable: Author, Title Date, Publisher, Edition or Volume, Pertinent Pages)
	U	
	V	
	W	
	X	

*A copy of this reference is not being furnished with this Office action. (See MPEP § 707.05(a).)
Dates in MM-YYYY format are publication dates. Classifications may be US or foreign.

EXHIBIT A

Search Notes

Application/Control No.	Applicant(s)/Patent under Reexamination	
10/607,330	MALNOE ET AL.	
Examiner	**Art Unit**	
DEBORAH A. DAVIS	1655	

SEARCHED			
Class	Subclass	Date	Examiner
NONE	N/A		DAD

INTERFERENCE SEARCHED			
Class	Subclass	Date	Examiner

SEARCH NOTES (INCLUDING SEARCH STRATEGY)		
	DATE	EXMR
UPDATED SEARCH	8/4/2008	DAD

Exhibit B

EXHIBIT B

UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450
www.uspto.gov

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
10/607,330	06/26/2003	Armand Malnoe	115808-365	4205

29157 7590 02/05/2009

BELL, BOYD & LLOYD LLP
P.O. Box 1135
CHICAGO, IL 60690

EXAMINER
DAVIS, DEBORAH A

ART UNIT	PAPER NUMBER
1655	

NOTIFICATION DATE	DELIVERY MODE
02/05/2009	ELECTRONIC

Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Notice of the Office communication was sent electronically on above-indicated "Notification Date" to the following e-mail address(es):

PATENTS@BELLBOYD.COM

EXHIBIT B

Advisory Action Before the Filing of an Appeal Brief	**Application No.** 10/607,330	**Applicant(s)** MALNOE ET AL.	
	Examiner DEBORAH A. DAVIS	**Art Unit** 1655	

--The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

THE REPLY FILED 12 November 2008 FAILS TO PLACE THIS APPLICATION IN CONDITION FOR ALLOWANCE.

1. ☒ The reply was filed after a final rejection, but prior to or on the same day as filing a Notice of Appeal. To avoid abandonment of this application, applicant must timely file one of the following replies: (1) an amendment, affidavit, or other evidence, which places the application in condition for allowance; (2) a Notice of Appeal (with appeal fee) in compliance with 37 CFR 41.31; or (3) a Request for Continued Examination (RCE) in compliance with 37 CFR 1.114. The reply must be filed within one of the following time periods:
   a) ☒ The period for reply expires 3 months from the mailing date of the final rejection.
   b) ☐ The period for reply expires on: (1) the mailing date of this Advisory Action, or (2) the date set forth in the final rejection, whichever is later. In no event, however, will the statutory period for reply expire later than SIX MONTHS from the mailing date of the final rejection.

   Examiner Note: If box 1 is checked, check either box (a) or (b). ONLY CHECK BOX (b) WHEN THE FIRST REPLY WAS FILED WITHIN TWO MONTHS OF THE FINAL REJECTION. See MPEP 706.07(f).

Extensions of time may be obtained under 37 CFR 1.136(a). The date on which the petition under 37 CFR 1.136(a) and the appropriate extension fee have been filed is the date for purposes of determining the period of extension and the corresponding amount of the fee. The appropriate extension fee under 37 CFR 1.17(a) is calculated from: (1) the expiration date of the shortened statutory period for reply originally set in the final Office action; or (2) as set forth in (b) above, if checked. Any reply received by the Office later than three months after the mailing date of the final rejection, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

NOTICE OF APPEAL

2. ☐ The Notice of Appeal was filed on _____. A brief in compliance with 37 CFR 41.37 must be filed within two months of the date of filing the Notice of Appeal (37 CFR 41.37(a)), or any extension thereof (37 CFR 41.37(e)), to avoid dismissal of the appeal. Since a Notice of Appeal has been filed, any reply must be filed within the time period set forth in 37 CFR 41.37(a).

AMENDMENTS

3. ☐ The proposed amendment(s) filed after a final rejection, but prior to the date of filing a brief, will not be entered because
   (a) ☐ They raise new issues that would require further consideration and/or search (see NOTE below);
   (b) ☐ They raise the issue of new matter (see NOTE below);
   (c) ☐ They are not deemed to place the application in better form for appeal by materially reducing or simplifying the issues for appeal; and/or
   (d) ☐ They present additional claims without canceling a corresponding number of finally rejected claims.

   NOTE: _____. (See 37 CFR 1.116 and 41.33(a)).
4. ☐ The amendments are not in compliance with 37 CFR 1.121. See attached Notice of Non-Compliant Amendment (PTOL-324).
5. ☐ Applicant's reply has overcome the following rejection(s): _____.
6. ☐ Newly proposed or amended claim(s) _____ would be allowable if submitted in a separate, timely filed amendment canceling the non-allowable claim(s).
7. ☐ For purposes of appeal, the proposed amendment(s): a) ☐ will not be entered, or b) ☐ will be entered and an explanation of how the new or amended claims would be rejected is provided below or appended.

   The status of the claim(s) is (or will be) as follows:
   Claim(s) allowed: _____.
   Claim(s) objected to: _____.
   Claim(s) rejected: _____.
   Claim(s) withdrawn from consideration: _____.

AFFIDAVIT OR OTHER EVIDENCE

8. ☐ The affidavit or other evidence filed after a final action, but before or on the date of filing a Notice of Appeal will not be entered because applicant failed to provide a showing of good and sufficient reasons why the affidavit or other evidence is necessary and was not earlier presented. See 37 CFR 1.116(e).
9. ☐ The affidavit or other evidence filed after the date of filing a Notice of Appeal, but prior to the date of filing a brief, will not be entered because the affidavit or other evidence failed to overcome all rejections under appeal and/or appellant fails to provide a showing a good and sufficient reasons why it is necessary and was not earlier presented. See 37 CFR 41.33(d)(1).
10. ☐ The affidavit or other evidence is entered. An explanation of the status of the claims after entry is below or attached.

REQUEST FOR RECONSIDERATION/OTHER

11. ☒ The request for reconsideration has been considered but does NOT place the application in condition for allowance because: See Continuation Sheet.
12. ☐ Note the attached Information *Disclosure Statement*(s). (PTO/SB/08) Paper No(s). _____
13. ☐ Other: _____.

/Christopher R. Tate/
Primary Examiner, Art Unit 1655

Continuation of 11. does NOT place the application in condition for allowance because: Applicant argues that the reference of Anatharaman et al , was withdrawn in the Non-Final Office Action dated February 5, 2008 and that since the newly applied reference of Anatharaman et al (i.e. different patent number) is a continuation, it would be improper to apply it again because both references share the same disclosure. This argument has been fully considered but not found to be persuasive of error. The examiner did not withdraw the reference of Anatharaman et al in the February 5, 2008 rejection. Applicant argues that the reference of Anatharaman et al does not teach chicory comprising sesquiterpene lactones in a concentration of at least 0.5% by weight as stated in the Office Action. Applicant argues that the reference of Anatharaman et al teaches the need to destroy or remove the lactones. Applicant argues that the final mixture is analyzed and was found to be free of lactones. These arguments have been fully considered but not found to be persuasive of error. In response, the reference of Anatharaman et al. teaches the addition of santonin to the mixture of chicory. Santonin is a type of sesquiterpene lactone. Also, the final mixture was analyzed by HPLC for bound sesquiterpene lactones (i.e. not free lactones) and none were found. Therefore, the reference of Anatharaman et al. still anticipates the instant claims because sesquiterpene lactones are found in the composition of chicory. Applicant argues that the reference of Anatharaman et al. does not disclose or suggest a thermally extruded plant material that includes more than one phytochemical agents capable of inhibiting at least one enzymatic and transcriptional activity of inhibit inflammation in a mammal. This argument has been fully considered but not found to be persuasive of error. In response, because there is not a difference in the cited composition and the instant claims, both teach thermally extruded chicory plant material, therefore, functional effects of inhibiting at least one of the enzymatic and transcriptional activity and inflammation in a mammal would be inherent. Applicant argues that the reference of Hwang is not combinable with the reference of Anatharaman et al. because Anatharaman's intent was to destroy or remove sesquiterpenes compounds present in the plant material but the reference of Hwang is directed toward the use of sesquiterpene compounds. This argument has been fully considered but not found to be persuasive for reasons disclosed in the above arguments that explained the reference of Anatharaman did not entirely remove sesquiterpene compounds but also added them to the composition. Therefore, for reasons provided above and of record, the current rejections are hereby maintained.

Exhibit C

EXHIBIT C

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant(s):	Malnoe et al.
Appl. No.:	10/607,330
Conf. No.:	4205
Filed:	June 26, 2003
Title:	COMPOSITIONS AND METHODS AGAINST INFLAMMATORY PROCESSES
Art Unit:	1654
Examiner:	D. Davis
Docket No.:	115808-365

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

RESPONSE TO FINAL OFFICE ACTION

Sir:

This Response is submitted in reply to the final Office Action dated August 15, 2008.

REMARKS

This Response is submitted in reply to the final Office Action dated August 15, 2008. No fee is due in connection with this Response. The Director is authorized to charge any additional fees which may be required, or to credit any overpayment to Deposit Account No. 02-1818. If such a withdrawal is made, please indicate the Attorney Docket No. 115808-365 on the account statement.

Claims 1, 3-4, 6, 8, 10-11, 14, 16 and 23-64 are pending in this application. Claims 2, 5, 7, 9, 12-13, 15, 17 and 17-22 were previously canceled. Claims 23-62 were previously withdrawn. In the Office Action, Claims 1, 4, 6, 8 and 10 are rejected under 35 U.S.C. §102. Claims 3, 11, 16 and 63-64 are rejected under 35 U.S.C. §103. For the reasons set forth below, Applicants respectfully submit that the rejections should be withdrawn.

In the Office Action, Claims 1, 4, 6, 8 and 10 are rejected under 35 U.S.C. 102(b) as being anticipated by U.S. Patent 6,197,361 to Anantharaman et al. ("*Anantharaman*"). Initially, Applicants note that the previous rejections of the claims under 35 U.S.C. §102(b) as being anticipated by U.S. Patent No. 5,592,033 to Anaantharaman et al. ("*Anantharaman II*") were withdrawn in the Non-Final Office Action dated February 5, 2008. Since *Anantharaman* is a continuation of *Anantharaman II*, the two references share the same disclosure. As such, Applicants submit that the current rejection in view of *Anantharaman* is also improper and respectfully traverse it for at least the reasons set forth below.

Independent Claim 1 recites, in part, a thermally extruded plant material that includes one or more phytochemical agents capable of inhibiting at least one of enzymatic and transcriptional activity to inhibit inflammation in a mammal. For example, Applicants have surprisingly discovered that upon thermal extruding, certain plants and/or plant extracts thereof can be generated with enhanced inhibition of enzyme activity and/or transcription activity in mammals which is believed to reduce the risk of inflammation. See, specification, page 5, lines 23-30. Thus, structurally the present claims require, in part, a plant material thermally extruded to inhibit at least one of enzymatic and transcriptional activity to treat inflammation. These are significant structural advantages, considering that the inflammation inhibiting nature of thermally extruded plant material of the present claims is a unique aspect of the invention. In

contrast, Applicants respectfully submit that *Anantharaman* fails to disclose or suggest every element of Claim 1.

Applicants respectfully submit that *Anantharaman* fails to disclose or suggest every element of Claim 1. For example, Applicants respectfully submit that *Anantharaman* fails to disclose or suggest a composition comprising one or more phytochemical agents capable of inhibiting at least one of enzymatic and transcriptional activity to inhibit inflammation in a mammal, wherein the one or more phytochemical agents is selected from the group consisting of sesquiterpene lactones, prebiotic fibers, dietary agents, and combinations thereof. Applicants respectfully disagree with the Patent Office's assertion that *Anantharaman* discloses that "chicory comprise of sesquiterpene lactones in a concentration of at least 0.5% by weight." See, Office Action, page 3, lines 4-6. The Patent Office cites column 1, lines 62-67; column 2, lines 21-65 and claims 1-7 of *Anantharaman* to support this statement. However, *Anantharaman* teaches the need to destroy or remove these sesquiterpene lactones. See, *Anantharaman,* col. 6, lines 30-34. Furthermore, contrary to the Patent Office's assertion, the sections of *Anantharaman* cited by the Patent Office does not disclose that the sesquiterpene lactones are still present. In fact, the final mixture is analyzed and "[n]o sesquiterpene lactones are detected". See, *Anantharaman,* col. 7, lines 29-31. The sesquiterpene lactones revealed in the analysis were only in the chicory starting ingredient and not in the final mixture. See, *Anantharaman,* col. 7, lines 31-34. Therefore, *Anantharaman* fails to disclose sesquiterpene lactones present in the extract.

Applicants further submit that *Anantharaman* fails to disclose or suggest a thermally extruded plant material that includes one or more phytochemical agents capable of inhibiting at least one of enzymatic and transcriptional activity to inhibit inflammation in a mammal. As discussed above, *Anantharaman* fails to disclose sesquiterpene lactones present in the extract capable of inhibiting at least one of enzymatic and transcriptional activity to inhibit inflammation in a mammal. Therefore, *Anantharaman* must also fail to disclose thermally treating the plant material in order to inhibit inflammation.

As discussed previously, the present claims require, in part, a plant material that is thermally extruded to inhibit at least one of enzymatic and transcriptional activity to inhibit inflammation in a mammal. By contrast, *Anantharaman* discloses a composition without any sesquiterpene lactones left in the composition to inhibit cyclooxygenase activity to inhibit

inflammation in a mammal. These are significant structural differences, considering that the inflammation inhibiting nature of the present claims is a unique aspect of the invention and is not disclosed or suggested by *Anantharaman*. For the reasons discussed above, Applicants respectfully submit that Claims 1 and Claims 4, 6, 8 and 10 that depend from Claim 1 are novel, non-obvious and distinguishable over *Anantharaman*.

Accordingly, Applicants respectfully request that the rejection of Claims 1, 4, 6, 8 and 10 under 35 U.S.C. §102 be withdrawn.

In the Office Action, Claims 1, 3-4, 6, 8, 10-11, 14, 16, 18 and 63-64 are rejected under 35 U.S.C. 103(a) as being unpatentable over *Anantharaman* in view of U.S. Patent 5,905,089 to Hwang et al.("*Hwang*"). Applicants respectfully submit that this rejection is improper and respectfully traverse it for at least the reasons set forth below.

Independent Claim 1 requires, in part, a thermally extruded plant material that includes one or more phytochemical agents capable of inhibiting at least one of enzymatic and transcriptional activity to inhibit inflammation in a mammal. Similarly, independent Claim 11 requires, in part, a thermally extruded plant material that includes one or more phytochemical agents capable of inhibiting at least one of enzymatic and transcriptional activity to treat inflammation in a mammal. Furthermore, independent Claim 63 requires, in part, a thermally extruded plant material, the active fragment including α-methylene-γ-butyrolactone, wherein the active fragment in an effective amount is capable of inhibiting at least one of enzyme and transcriptional activity to inhibit inflammation. Applicants have surprisingly discovered that upon thermal extruding certain plants and/or plant extracts thereof can be generated with enhanced inhibition of enzyme activity and/or transcription activity in mammals which is believed to reduce the risk of inflammation. See, specification, page 5, lines 23-30. In contrast, Applicants respectfully submit that there exists no reason why the skilled artisan would combine the cited references to arrive at the present claims.

As discussed above, structurally, the present claims require, in part, a plant material thermally extruded to inhibit at least one of enzymatic and transcriptional activity to treat inflammation. Applicants have surprisingly discovered that upon thermal extruding, certain plants and/or plant extracts thereof can be generated with enhanced inhibition of enzyme activity and/or transcription activity in mammals which is believed to reduce the risk of inflammation. See, specification, page 5, lines 23-30. These are significant structural differences, considering

that the inflammation inhibiting nature of thermally processed plant material of the present claims is a unique aspect of the invention and is not disclosed or suggested in the prior art.

Applicants respectfully submit that the skilled artisan would have no reason to combine the cited references to obtain the present claims because the cited references teach away from each other and the claimed invention. For example, as previously discussed, *Anantharaman* discloses destroying or removing sesquiterpene lactones. Specifically, *Anantharaman* teaches the need to "destroy or remove" these sesquiterpene lactones. See, *Anantharaman,* col. 6, lines 30-34. In contrast, *Hwang* is entirely directed toward the use of sesquiterpene lactones obtained from plant material. See, *Hwang*, Abstract.

Further, references are not properly combinable or modifiable if their intended purpose is destroyed. For instance, if the proposed modification would render the prior art invention being modified unsatisfactory for its intended purpose, then there is no suggestion or motivation to make the proposed modification. *In re Gordon*, 733 F.2d 900, 221 USPQ 1125 (Fed. Cir. 1984). This is exactly the case where *Anantharaman* is directed toward a matrix making up a cereal product that must be gelatinized in order to "remove or destroy the sesquiterpene compounds" present in the plant material, see, *Anantharaman,* col. 6, lines 30-34, and where *Hwang* is entirely directed toward the use of sesquiterpene lactones obtained from plant material, see, *Hwang*, Abstract.

Applicants respectfully submit that the claims must be viewed as a whole as defined by the claimed invention and not dissected into discrete elements to be analyzed in isolation. *W.L. Gore & Assoc., Inc. v. Garlock, Inc.*, 721 F.2d 1540, 1548, 220 USPQ 303, 309 (Fed. Cir. 1983); *In re Ochiai*, 71 F.3d 1565, 1572, 37 USPQ2d 1127, 1133 (Fed. Cir. 1995). Further, one should not use hindsight reconstruction to pick and choose among isolated disclosures in the prior art to deprecate the claimed invention. *In re Fine*, 837 F.2d at 1075. (Fed. Cir. 1988).

For at least the reasons discussed above, the skilled artisan would have no reason to combine *Anantharaman* and *Hwang* to arrive at the present claims. Applicants respectfully submit that the combination of *Anantharaman* and *Hwang* is improper.

Accordingly, Applicants respectfully request that the obviousness rejection with respect to Claims 3, 11, 16 and 63-64 be reconsidered and withdrawn.

For the foregoing reasons, Applicants respectfully request reconsideration of the above-identified patent application and earnestly solicit an early allowance of same. In the event there remains any impediment to allowance of the claims which could be clarified in a telephonic interview, the Examiner is respectfully requested to initiate such an interview with the undersigned.

Respectfully submitted,

BELL, BOYD & LLOYD LLP

BY _____

Robert M. Barrett
Reg. No. 30,142
Customer No. 29157
Phone No. 312-807-4204

Dated: November 12, 2008

Exhibit D

EXHIBIT D

(12) **United States Patent**
Anantharaman et al.

(10) **Patent No.: US 6,197,361 B1**
(45) **Date of Patent: *Mar. 6, 2001**

(54) **GELATINIZED CEREAL PRODUCT CONTAINING OLIGOSACCHARIDE**

(75) Inventors: **Helen Gillian Anantharaman**, Bridgewater, CT (US); **Olivier Ballevre**, Lausanne; **Florence Rochat**, Montreux, both of (CH)

(73) Assignee: **Nestec S.A.**, Vevey (CH)

(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 0 days.

This patent is subject to a terminal disclaimer.

(21) Appl. No.: **09/375,105**

(22) Filed: **Aug. 16, 1999**

Related U.S. Application Data

(63) Continuation of application No. 08/980,714, filed on Dec. 1, 1997, now Pat. No. 5,952,033.

(30) **Foreign Application Priority Data**

Dec. 24, 1996 (EP) 96203705
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(58) **Field of Search** 426/560, 618, 426/623, 449, 805

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Primary Examiner—Nina Bhat
(74) *Attorney, Agent, or Firm*—Bell, Boyd & Lloyd LLC

(57) **ABSTRACT**

A gelatinized cereal product which contains a plant material which is a source of inulin; for example chicory. Sufficient of the plant material is included to provide at least about 0.25% by weight of inulin on a dry basis. The cereal product may be used as a pet food or breakfast cereal.

16 Claims, No Drawings

GELATINIZED CEREAL PRODUCT CONTAINING OLIGOSACCHARIDE

This a continuation of U.S. patent application Ser. No. 08/980,714 filed on Dec. 1, 1997, now U.S. Pat. No. 5,952,033.

FIELD OF THE INVENTION

This invention relates to gelatinized cereal product which contains oligosaccharide in the form of inulin; especially in the form of natural sources of inulin such as chicory. In sue, the cereal product has a beneficial effect in the gastro-intestinal tract of the consumer and hence upon the consumer as a whole. The invention also relates to a process of producing the cereal product and to methods of promoting beneficial effects in the gastro-intestinal tracts of humans and animals. The cereal product is particularly suitable for use as a pet food.

BACKGROUND OF THE INVENTION

Oligosaccharides such as inulin and various fructo-oligosaccharides are reported to promote the growth of bifido- and lacto-bacteria in the gastro-intestinal tract at the expense of pathogens such as *Clostridium perfringens.* For example, see Gibson et al; 1994; *Food Microbiology,* 11(6), 491–498. Although most reported experimentation has been carried out in vitro, there have been reports that these oligosaccharides have a similar effect in the gut of rats and humans. Further, promoting the growth of bifido- and lacto-bacteria through the use of oligosaccharides is reported to have various beneficial effects on animals and humans. These beneficial effects include the prevention or treatment of diarrhea, and increased growth; improved ability to breed, and enhanced health.

These beneficial effects have resulted in use of oligosaccharides, particularly fructo-oligosaccharides, in human foods and feeds for live stock. For example, International patent application WO 94/27617 discloses the use of a caramel which contains fructo-oligosaccharides in animal feed to enhance the health of live stock. However, reports of the use of inulin also exist. For example Japanese patent application 63-309147 discloses the use of a purified inulin in the feed for younger animals to prevent diarrhea after weaning and to increase body weight. Similarly, U.S. Pat. No. 4,865,852 discloses the use of inulin in the form of treated chicory as a feed for live stock.

Although the primary focus for oligosaccharides has been human foods and feeds for live stock, the use of fructo-oligosaccharides in a veterinary diet for pets has also been suggested (Willard et al; 1994, *Am. J. Vet. Res.,* 55, 654–659). Further, products containing fructo-oligosaccharide are on the market; for example the Eukanuba product (The IAMS Company).

For the products which contain inulin, the inulin is usually purified from plants which contain higher concentrations of inulin; such as chicory, Jerusalem artichoke, leek and asparagus. Otherwise, the plant material is treated in some form or another prior to use. A reason for the purification or treatment is that the plants themselves are reported to have bitter flavors which result in palatability problems; see for example U.S. Pat. No. 4,865,852. This is particularly the case with chicory where the bitter flavors are believed to be due to the concentrations of sesquiterpene lactones such as lactucin and lactucopicrin in chicory. Also, it is generally believed that more accurate control of the amounts added may be obtained with purified product. Various procedures

for purifying the inulin or treating the plant material have been reported. Usually however they include the steps of chopping up the plant, extracting it, and hydrolyzing it with acids or enzymes. The hydrolysate is then collected and condensed to obtain the inulin. For example, Japanese patent application 63-309147 disclosed grinding chicory tubers, partially hydrolyzing them with acids, and then drying the hydrolysate with or without neutralization.

Unfortunately, fructo-oligosaccharides and purified inulin greatly add to the cost of the products. Consequently, for pet foods, their use has been confined to specialty veterinary products such as the Eukanuba product and to pet treats. Similarly, for human foods, their use has been confined to specialty products.

Therefore there is a need for a cereal product which has the properties of food which contain fructo-oligosaccharides and purified inulin, which is palatable to humans and animals, and which may be inexpensively produced.

SUMMARY OF THE INVENTION

Accordingly, in one aspect, this invention provides a cereal product which comprises a gelatinized starch matrix which contains an amount of a plant material which is a source of inulin, sufficient to provide at least about 0.25% by weight inulin, on a dry matter basis.

It has been surprisingly found that adding a natural plant material which is a source of inulin to the usual ingredients of gelatinized cereal products and then gelatinizing the ingredients does not adversely affect the palatability of the food to humans and pets. This is despite the presence of sesquiterpene lactones such as lactucin and lactucopicrin in the plant materials. The gelatinisation of the ingredients of the cereal products surprisingly appears to remove or destroy these compounds. Also, trials indicate that dogs may find the cooked food even more palatable than commercially available foods. Given that these plant materials were thought to be highly unpalatable to animals, this result is extremely surprising. Cats find the cooked food at least as palatable as commercially available foods. It is also surprisingly found that gelatinizing of the ingredients does not result in any significant degradation of the shorter chain oligosaccharides of inulin. Therefore, it is believed that the gelatinized cereal product retains the properties of unprocessed inulin.

The plant material preferably comprises an inulin-rich plant material such as chicory or Jerusalem artichoke, or both; especially chicory. The gelatinized cereal product preferably contains sufficient of the plant material such that it comprises at least 0.5% by weight of inulin on a dry matter basis. The maximum amount is inulin is preferably about 10% by weight on a dry matter basis. The gelatinized cereal product preferably includes at least about 0.01% by weight of kestose; 0.01% by weight of nystose and 0.01% by weight of fructosyl-nystose. More preferably kestose, nystose and fructosyl-nystose make up at least about 0.1% by weight of the gelatinized cereal product; for example the gelatinized cereal product may include at least about 0.04% by weight of kestose; 0.04% by weight of nystose and 0.04% by weight of fructosyl-nystose.

Preferably, the gelatinized matrix further includes protein. The gelatinized cereal product preferably comprises an extrusion cooked product. The extrusion cooked product may be in dried pellet form, dried expanded form, or flaked form.

In a further aspect, this invention provides a process of preparing a gelatinized cereal product which contains at

least about 0.25% by weight of inulin on a dry matter basis, the process comprising gelatinizing a starch source, a protein source, and a plant material which is a source of inulin to form a gelatinized starch and protein matrix which contains the inulin.

Preferably the starch source, protein source, and plant material are extrusion cooked and then extruded. Further, the extrudate may be dried.

In another aspect, this invention provides a method of increasing the digestibility of a cereal product comprising incorporating a plant material which is a source of inulin into the cereal product.

In a yet further aspect, this invention provides a method of decreasing fecal volume of a pet, the method comprising feeding the pet a gelatinized cereal product which contains an amount of a plant material which is a source of inulin, sufficient to provide at least about 0.25% by weight inulin, on a dry matter basis.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

Embodiments of the invention are now described, by way of example only.

The invention provides a gelatinized cereal product which contains an amount of a plant material, which is a source of inulin, sufficient to provide at least about 0.25% by weight inulin, on a dry matter basis. The plant material used may be any suitable source of inulin; for example chicory, Jerusalem artichoke, leek, onion, yacon, asparagus which contains high levels or inulin, and mixtures of these plants. However inulin rich plant materials such as chicory and Jerusalem artichoke are preferred; especially chicory. These plant materials usually comprise at least about 50% by weight of inulin. For ease of handling, the plant material is preferably in a dried, comminuted form. For simplicity of description, the processes described below are described with reference to the use of dried, comminuted chicory. However it is to be understood that any suitable plant material may be used in any suitable form.

The remaining ingredients included in the gelatinized cereal product may be any suitable ingredients commonly used in gelatinized cereal products. Usually these ingredients include a starch source and a protein source. Suitable starch sources are, for example, grains such as corn, rice, wheat, beets, barley, oats, soy, and mixtures of these. Suitable protein sources may be selected from any suitable animal or vegetable protein source; for example meat meal, bone meal, fish meal, soy protein concentrates, milk proteins, gluten, and the like. The choice of the starch and protein sources will be largely determined by the nutritional needs of the animal or human, palatability considerations, and the type of cereal product produced. Various other ingredients, for example, sugar, salt, spices, seasonings, vitamins, minerals, flavoring agents, fats and the like may also be incorporated into the gelatinized cereal product as desired.

The gelatinized cereal product may be produced in many different ways as desired. However, for a dried cereal product, an especially suitable way of producing the product is extrusion cooking. This may be done as is well known in the art. For example, in one suitable process, a feed mixture is fed into a preconditioner. The feed mixture is primarily made up of a starch source, a protein source, and the chicory. Preferably the chicory comprises at least about 1% by weight of the feed material; more preferably at least about 2% by weight. The maximum of chicory is preferably about 20% by weight; especially about 10% by weight.

In the preconditioner, water or steam, or both, is mixed into the feed mixture. Sufficient water or steam is mixed into the feed mixture to moisten the feed mixture. If desired, the temperature of the feed mixture may be raised in the preconditioner to about 60° C. to about 90° C. by weight. A suitable preconditioner is described in U.S. Pat. No. 4,752,139. Plainly, it is not necessary to use a preconditioner.

The moistened feed leaving the preconditioner is then fed into an extruder. The extruder may be any suitable single or twin screw, cooking-extruder. Suitable extruders may be obtained from Wenger Manufacturing Inc, Clextral SA B ühler AG, and the like. During passage through the extruder, the moistened feed passes through a cooking zone, in which it is subjected to mechanical shear and is heated; for example up to a maximum temperature of up to about 150° C., and a forming zone. The gauge pressure in the forming zone is about 300 kPa to about 10 MPa as desired. If desired, water or steam, or both, may be introduced into the cooking zone. During passage through the extruder, the starch source of the moistened feed is gelatinized to provide a gelatinized matrix structure primarily of starch, protein and chicory.

The gelatinized matrix leaving the extruder is forced through a suitable die; for example a die as described in European patent application 0665051; the disclosure of which is incorporated by reference. A shaped extrudate, which has a cross-sectional shape corresponding to that of the orifice of the die, leaves the die. Depending upon the conditions in the extruder and the starch source used, the shaped extrudate expands to a greater or lesser extent. The shaped extrudate is then cut into pieces using blades. The individual pieces are then dried and, if desired, coated with protective or flavoring agents, or both. After cooling, the pieces may be packed into suitable packages. Alternatively, the individual pieces may be formed into flakes and then dried.

Depending upon the ingredients used, the gelatinized cereal product may be in the form of dried kibbles suitable for use as pet foods, expanded pieces suitable for use in breakfast cereals, flakes suitable for use in breakfast cereals, and the like.

It is also possible to produce a dried cereal product by mixing together water and the ingredients of cereal product; for example in a preconditioner. The wet mixture may then be shaped into a desired shape; for example using shaping rollers. The shaped mixture may then be baked in an oven; for example at about 220° C. to about 280° C. for about 10 minutes to about 1 hour. The dried cereal product has the appearance of a baked biscuit.

If it is desired to produce a simulated meat product which may be used in canned pet foods, the processes described in U.S. Pat. Nos. 4,781,939 and 5,132,137 may be used. In these processes, a protein source, especially a meat material, is emulsified. The meat material may be any suitable source of animal protein; for example the muscular or skeletal meat of mammals, poultry, and fish or meat by-products such as hearts, liver, kidneys, tongue and the like, or meat meals. Vegetable protein sources may also be included if desired. The exact composition may be selected according to cost and the desired flavor. The emulsification may be carried out in any suitable equipment.

The dried chicory is added to the emulsion. Also, if desired or needed, additional protein may be added to the emulsion. The additional protein may be any protein source as mentioned above. The exact choice will depend upon availability, cost and palatability. Usually about 5% to about 35% of the further protein source is used.

If desired or required, fats may also be added to the emulsion. Usually the amount of fat in the emulsion must be controlled to facilitate processing and to obtain an acceptable product. However, the meat material may well contain the desired amount of fats and hence adjustment may not be necessary. Typically at this stage the emulsion contains a maximum fat level of about 25% by weight. Conveniently, the amount of fat in the emulsion is in the range of about 5% to 15% by weight; more preferably about 7% to about 12% by weight. The mass ratio protein to fat in the emulsion is preferably about 1:1 to about 7:1. If added, the fats may be any suitable animal fats; for example tallow, or may be vegetable fats.

Additional ingredients such as sugars, salts, spices, seasonings, flavoring agents, minerals, and the like may also be added to the emulsion. The amount of additional ingredients used is preferably such that they make up about 1% to about 5% by weight of the gelatinized cereal product.

Water may also be added to provide from about 45% to 80% by weight moisture in the emulsion. If sufficient moisture is present in the meat material, water need not be added.

Once mixed, the emulsion is preferably fed through a vacuum stuffer, or similar de-aeration apparatus, to de-aerate the emulsion. This removes air which may otherwise cause disruption of the formulated emulsion product and reduce its meat-like appearance.

The emulsion is then fed to an emulsion mill which subjects the emulsion to rapid mechanical heating and shearing. Any suitable emulsion mill may be used, for example the emulsion mill disclosed in U.S. Pat. No. 5,132,137. Other suitable emulsion mills are commercially available under the trade name of Trigonal and may be obtained from Siefer Machinenfabrik GmbH & Co KG, Bahnhofstrasse 114, Postfach 101008, Velbert 1, Germany.

The temperature of the emulsion is raised to the desired coagulation temperature in the emulsion mill in a few seconds. For example, the temperature may be raised to from about 100° C. to about 120° C. Alternatively, the temperature may be raised to in the range of about 45° C. to about 75° C. as described in U.S. Pat. No. 5,132,137. Usually the mechanical energy generated in the emulsion mill will be sufficient to heat the emulsion to the desired temperature but this may be supplemented by the injection of superheated steam.

The heated emulsion leaving the emulsion mill is then transferred to a holding tube. In the holding tube, the heated emulsion coagulates while moving slowly along the holding tube. The residence time of the heated emulsion in the holding tube is sufficient for the emulsion to have coagulated into a firm emulsion product upon reaching the exit of the holding tube.

The firm emulsion product leaving the holding tube is then transferred to a cutter where it is cut into chunks of size suitable for use in a pet food. The chunks have the appearance and texture of meat. The chunks may be subjected to flaking if desired. The chunks may also be formulated into a chunk-in-gravy type of product.

Other procedures for producing chunks are known and may be used; for example extruding a feed mixture, cooking the feed mixture in a steam oven, and the cutting the cooked extrudate into chunks.

If it is desired to produce a canned pet food in the form of a meat loaf; a meat batter may be prepared by emulsifying a suitable meat material to produce a meat emulsion. The meat material may be any suitable meat source, for example as described above. Suitable gelling agents, for example gums such as kappacarrageenan, locust bean gum, guar gum and xanthan gum may be added to the meat emulsion. Usually no more than about 2% by weight of gum is needed. The dried chicory is then added to the meat emulsion.

Additional ingredients such as sugars, salts, spices, seasonings, flavoring agents, minerals, and the like may also be added to the meat emulsion. The amount of additional ingredients used is preferably such that they make up about 0.25% to about 5% by weight of the meat batter.

Water may also be added the meat emulsion to provide from about 70% to about 85% by weight. If sufficient moisture is present in the meat material, water need not be added.

The meat emulsion is then heated to a temperature above about 65° C. in a mixer-cooker. Steam may be injected into the meat batter if desired. The heated meat emulsion is then again emulsified to provide a loaf batter and the loaf batter maintained at a temperature above about 60° C. until filling into cans.

It will be appreciated that the gelatinized cereal product may be produced by any suitable process and not only those described above. Other types of oligosaccharides may also be included in the gelatinized cereal product; for example fructo oligosaccharide and soy oligosaccharide. The soy oligosaccharides may be added in the form of soy meal or other suitable soy source.

The cereal products may be in any suitable form; for example dried, semi-wet and wet. However, the matrix making up the cereal product must be gelatinized in order to remove or destroy the sesquiterpene compounds present in the inulin-containing plant material.

Specific examples are now described for further illustration.

EXAMPLE 1

A feed mixture is made up of about 58% by weight corn, about 5.5% by weight of corn gluten, about 22% by weight of chicken and fish meal, dried chicory and salts, vitamins and minerals making up the remainder. Two levels of chicory are used; about 2.5% and about 5%. Also, two commercial types of chicory are used; Leroux standard blend and Leroux Rubis variety. Both types are commercially available on the French market from the Leroux company.

The feed mixture is fed into a preconditioner and moistened. The moistened feed leaving the preconditioner is then fed into an extruder-cooker and gelatinized. The gelatinized matrix leaving the extruder is force through a die and extruded. The extrudate leaving the die head is cut into pieces suitable for feeding to cats, dried, and cooled to pellets.

The pellets are fed to a panel of 80 cats. For comparison, the cats may choose between the pellets with chicory an control pellets which are identical except that they do not contain chicory. The amount that each cat eats of each type of pellet is monitored. The results are as follows:

Example No	Chicory Type	Chicory level %	Percentage consuming pellets containing chicory
1A	Standard	2.5	42
1B	Standard	5.0	52

-continued

Example No	Chicory Type	Chicory level %	Percentage consuming pellets containing chicory
1C	Rubis	2.5	46
1D	Rubis	5.0	52

The results indicate that the pellets with chicory have substantially the same palatability as those without. However, even more surprisingly, as the chicory content increases, the palatability appears to increase.

The gut flora of the cats is analyzed and it is determined that bifidobacteria counts have increased while *C. perfringens* counts have decreased. Further, fecal pH and odors are found to have decreased. Energy and mineral digestibility have increased leading to a decrease in fecal volume.

The pellets are crushed and extracted with methanol by boiling under reflux for 1 hour. The extract is twice partitioned between water and chloroform and santonin is added. The chloroform phase is separated, dried and evaporated. The residue is dissolved in a mixture of methanol and chloroform and analyzed using HPLC for free sesquiterpene lactones. The water phase is run through a column and glycosylated compounds eluted from the column using methanol. The eluant is evaporated, dissolved in water and treated with cellulase at 40° C. for 2 hours. Santonin is added to the hydrolysate and the mixture extracted with ethyl acetate. The mixture is then analyzed using HPLC for bound sesquiterpene lactones.

No sesquiterpene lactones are detected. Similar analysis of the chicory starting ingredient reveals between 130 to 350 ppm free sesquiterpene lactones and between 380 to 680 ppm bound sesquiterpene lactones.

EXAMPLE 2

A trial is conducted using 30 dogs. The control food is the Friskies Menu Energy product, which is dried dog food available on the market. Two test foods are prepared; they correspond to the Friskies Menu Energy product except that they include 5% by weight of chicory. One test food contains the Leroux standard blend chicory and the other contains the Leroux Rubis chicory.

The foods are fed to the panel of 30 dogs. The amount that each dog eats of each type of food is monitored. The trial is then repeated. The results are as follows:

Example No	Chicory Type	Chicory level %	Percentage consuming pellets containing chicory
2A	Standard	5.0	62
2B			69
2C	Rubis	5.0	80
2D			89

The results indicate that the foods with chicory have improved palatability as compared to the control. In the case of the standard chicory, the improvement is pronounced.

The gut flora of the dogs is analyzed and it is determined that bifidobacteria counts have increased while *C. perfringens* counts have decreased. Further, fecal pH and odors are found to have decreased. Energy and mineral digestibility have increased leading to a decrease in fecal volume.

EXAMPLE 3

The amount of kestose, nystose and fructosyl-nystose in the pellets of example 1 is measured.

Oligo-saccharide	Example 1A	Example 1B	Example 1C	Example 1D
kestose	0.04	0.08	0.04	0.07
nystose	0.04	0.08	0.05	0.08
fructosyl-nystose	0.04	0.09	0.05	0.09
Total	0.12	0.25	0.14	0.24

The amount of total inulin, kestose, nystose and fructosyl-nystose in the two different types of chicory used in example 1 is measured. On the basis of this determination and the amount of chicory added, a theoretical amount of kestose, nystose and fructosyl-nystose is determined for each pellet. The theoretical, combined amounts of kestose, nystose and fructosyl-nystose in the pellets are then compared to the measured, combined amounts. The theoretical amounts and the measured amounts are comparable indicating that little or no degradation of the kestose, nystose and fructosyl-nystose has taken place during extrusion cooking.

EXAMPLE 4

A feed mixture is made up of rice flour, wheat flour, sugar, malt, vegetable fats, salt and about 5% by weight of chicory. The feed mixture is fed into a preconditioner and moistened. The moistened feed leaving the preconditioner is then fed into an extruder and gelatinized. The temperature at the exit of the extruder is about 150° C. The pressure in the extruder reaches about 130 bar. The gelatinized matrix leaving the extruder is forced through a die and extruded. The extrudate expands upon leaving the die head and is cut into pieces of about 2 to 3 mm. The pieces are then dried to a moisture content of about 1% by weight. The pieces are in the form of a puffed breakfast cereal.

The pieces are tasted by a panel of consumers and are found to have a good taste; comparable to a puffed breakfast cereal produced without chicory.

EXAMPLE 5

A trial is conducted using 16 dogs. The control food is the Friskies Menu Vitality product, which is dried dog food available on the market. A test food is prepared which correspond to the Friskies Menu Vitality product except that it include 3% by weight of Leroux standard blend chicory.

Eight dogs are fed the control food and eight dogs are fed the test food. Feces samples are collected from each dog, heated for 2 hours at 30° C., and the compounds released trapped on a Tenax tube. The trapped compounds are desorbed on a gas chromatograph. The levels of dimethylsulfide, dimethyldisulfide, and dimethyltrisulfide are determined as follows:

Food	Dimethylsulfide Area	Dimethyldisulfide Area	Dimethyltrisulfide Area
Control	5731312	1084439	379164
Test	1719824	48824	43739

The results indicate that the feces of the dogs fed the test diet have much reduced amounts of those sulfur containing compounds which are believe to cause unpleasant odors.

The levels of short chain fatty acids in the feces are also determined by gas chromatograph as follows:

Food	Acetate Area	Propionate Area	Butyrate Area	Total Area
Control	120	66	20	207
Test	159	91	18	267

The results indicate that the dogs fed the test diet have higher levels of short chain fatty acids in the gut. Short chain fatty acids are important nutrients for gut cells, and liver and muscles. An increase in the content of short chain fatty acids in the gut may result in an improvement in the health of the animal or human.

What is claimed is:

1. A dried cereal product comprising a gelatinized starch matrix which contains a plant material which is a source of inulin in an amount sufficient to provide at least about 0.25% by weight inulin, on a dry matter basis.

2. A dried cereal product according to claim 1 in which the gelatinized starch matrix includes protein.

3. A dried cereal product according to claim 1 which is in the form of expanded breakfast cereal pieces or breakfast cereal flakes.

4. A dried cereal product according to claim 1 in which the plant material is selected from the group consisting of chicory and Jerusalem artichoke.

5. A dried cereal product according to claim 1 which comprises at least 0.5% by weight of inulin, on a dry basis.

6. A dried cereal product according to claim 1 which comprises at least about 0.1% by weight of kestose, nystose and fructosyl-nystose.

7. A dried pet food which comprises a gelatinized starch matrix which contains at least about 0.25% by weight inulin, on a dry matter basis.

8. A dried pet food according to claim 7 in which the gelatinized starch matrix includes protein.

9. A dried pet food according to claim 7 which is a dried pellet.

10. A dried pet food according to claim 7 which comprises at least 0.5% by weight of inulin, on a dry basis.

11. A dried pet food according to claim 7 which comprises at least about 0.1% by weight of kestose, nystose and fructosyl-nystose.

12. A process of preparing a dried cereal product which contains at least about 0.25% by weight of inulin on a dry basis, the process comprising gelatinizing a starch source, a protein source, and a plant material to form a gelatinized starch and protein matrix which contains the inulin.

13. A process according to claim 12 in which the starch source, the protein source, and the plant material are extrusion cooked and then extruded.

14. A method for improving food digestibility in a pet, the method comprising feeding the pet a dried pet food in the form of a gelatinized starch matrix which contains a at least about 0.25% by weight inulin, on a dry matter basis.

15. A method for increasing the population density of lactic acid bacteria in the gastro-intestinal tract of a pet, the method comprising feeding the pet a dried pet food in the form of a gelatinized starch matrix which contains at least about 0.25% by weight inulin, on a dry matter basis.

16. A method of reducing pet fecal odors, the method comprising feeding the pet a dried pet food in the form of a gelatinized starch matrix which contains at least about 0.25% by weight inulin, on a dry matter basis.

* * * * *

Exhibit E

US005905089A

United States Patent [19]

Hwang et al.

[11] Patent Number: 5,905,089

[45] Date of Patent: May 18, 1999

[54] **USE OF SESQUITERPENE LACTONES FOR TREATMENT OF SEVERE INFLAMMATORY DISORDERS**

[75] Inventors: **Daniel H. Hwang; Nikolaus H. Fischer**, both of Baton Rouge, La.

[73] Assignee: **Board of Supervisors of Louisiana State University and Agricultural and Mechanical College**, Baton Rouge, La.

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[52] **U.S. Cl.** **514/468**

[58] **Field of Search** 514/468

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A. Fein, "Treatment of Severe Systemic Inflammatory Response Syndrome and Sepsis with a Novel Bradykinin Antagonist, Deltibant (CP–0127)," *J. Am. Med. Assoc.*, vol. 277, pp. 482–487 (1997).

P. Chanmugam, "Radicicol, a Protein Tyrosine Kinase Inhibitor, Suppresses the Expression of Mitogen–Inducible Cyclooxygenase in Macrophages Stimulated with Lipopolysaccharide and in Experimental Glomerulonephritis," *J. Biol. Chem.*, vol. 270, pp. 5418–5426 (1995).

A. Novogrodsky et al., "Prevention of Lipopolysaccharide–Induced Lethal Toxicity by Tyrosine Kinase Inhibitors," *Science*, vol. 264, pp. 1319–1322 (1994).

T. Akiyama et al., "Genistein, a Specific Inhibitor of Tyrosine–Specific Protein Kinases," Journal of Biological Chemistry, vol. 262, pp. 5592–5595 (1986).

Hwang et al., "Inhibition of the Expression of Inducible Cyclooxygenase and Proinflammatory Cytokines by Sesquiterpene Lactones in Macrophages Correlates with the Inhibition of MAP Kinases," *Biochem. and Biophys. Res. Comm.*, vol. 226, pp. 810–818 (1996).

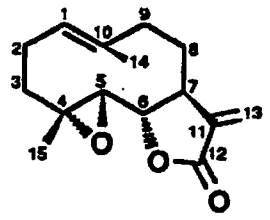
Primary Examiner—Keith D. MacMillan

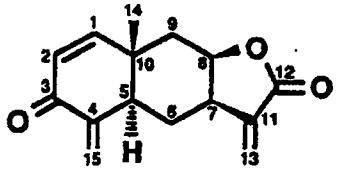
Attorney, Agent, or Firm—Bonnie J. Davis; John H. Runnels

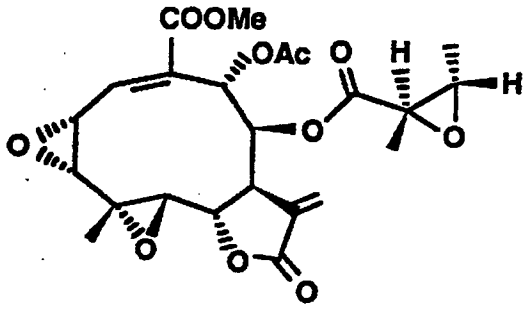
[57] **ABSTRACT**

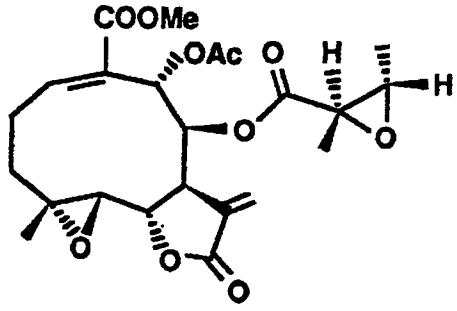
Sesquiterpene lactones are useful in suppressing the early proinflammatory cytokines, and in ameliorating septic shock and other severe inflammatory disorders. Sesquiterpene lactones with an α-methylene-γ-lactone functional group suppress the expression of the inducible cyclooxygenase-2 and proinflammatory cytokines (Interleukin-1α and β, IL-1, and tumor necrosis factor-α (TNFα)) in mammalian macrophages stimulated with lipopolysaccharide. This suppression correlated with the inhibition of protein-tyrosine phosphorylation including the mitogen-activated protein kinases.

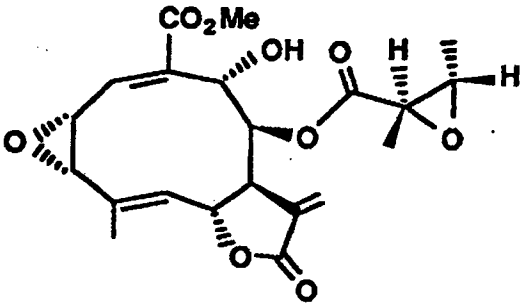
24 Claims, 6 Drawing Sheets

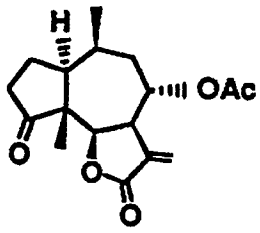
(1) 

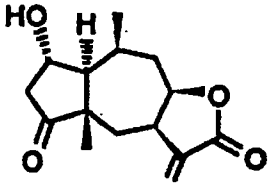
(2) 

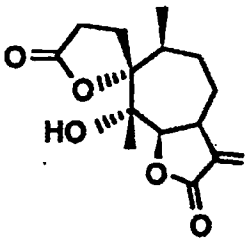
(3) 

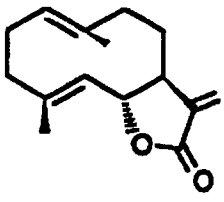
(4) 

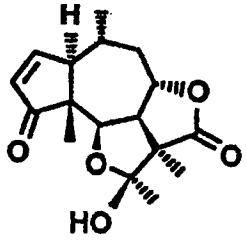
(5) 

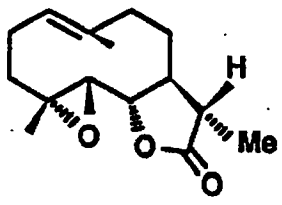
(6) 

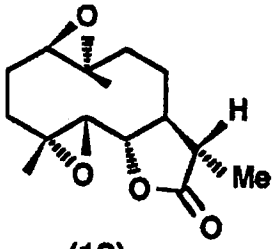
(7) 

(8) 

(9) 

(10) 

(11) 

(12) 

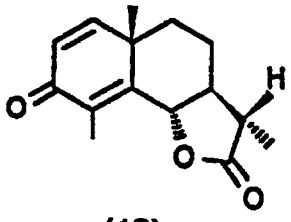
(13) 

FIGURE 1

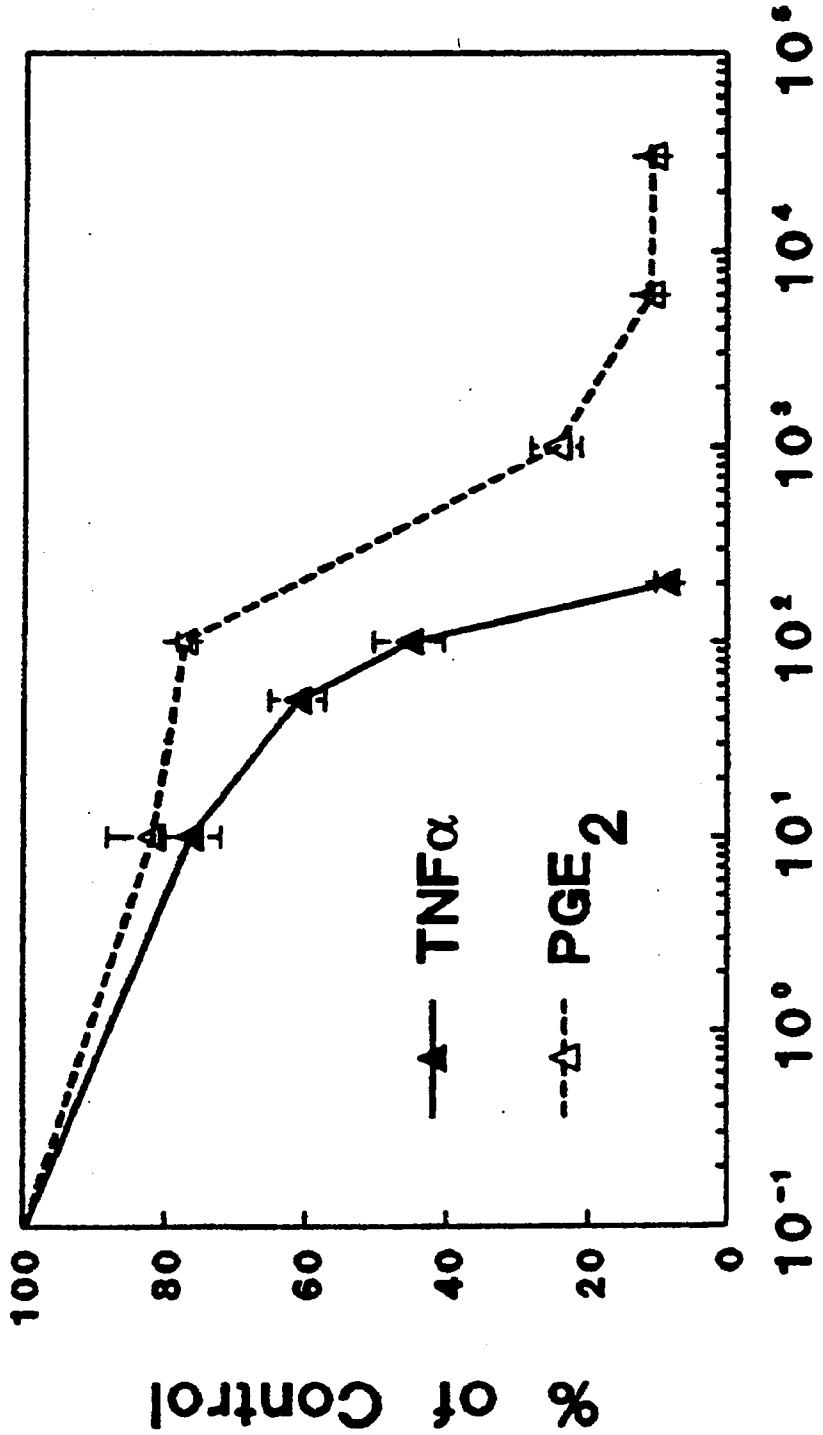

% of Control
100
80
60
40
20
0
TNFα
PGE2
10⁻¹
10⁰
10¹
10²
10³
10⁴
10⁵
Parthenolide (ng/ml)

FIGURE 2

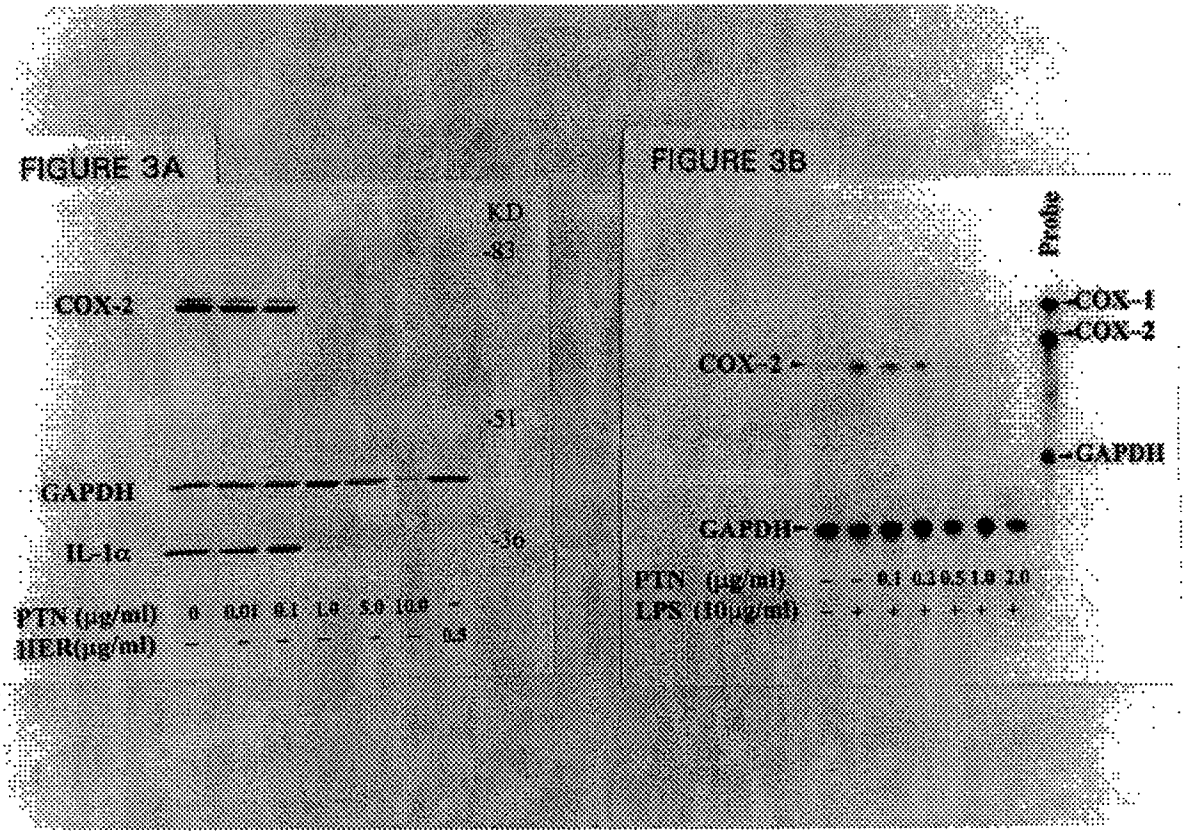


FIGURE 3A
KD
-83
COX-2
-51
GAPDH
IL-1α
-36
PTN (μg/ml) 0 0.01 0.1 1.0 5.0 10.0 –
HER(μg/ml) – – – – – – 0.5
FIGURE 3B
Probe
COX-1
COX-2
COX-2 – – – – –
GAPDH
GAPDH
PTN (μg/ml) – – 0.1 0.3 0.5 1.0 2.0
LPS (10μg/ml) – + + + + + +

FIGURE 4A

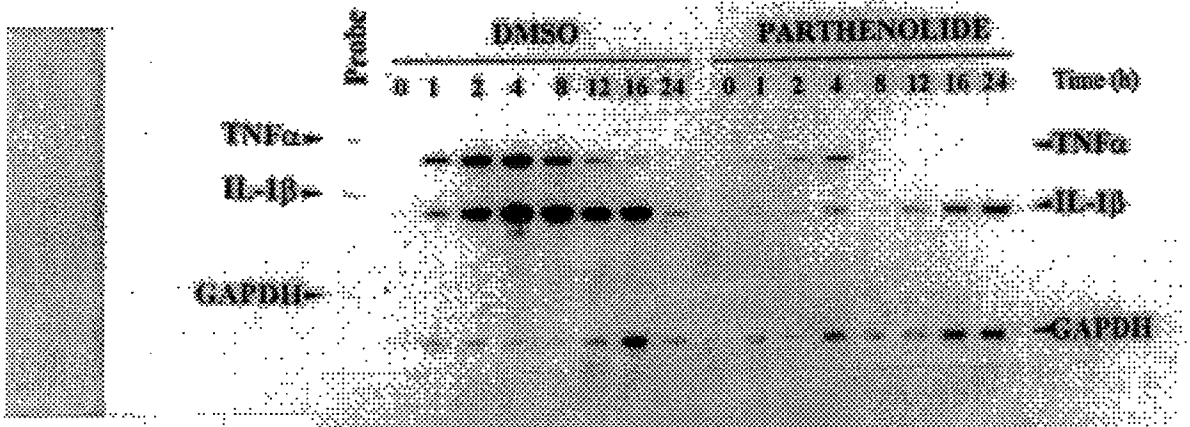


FIGURE 4B

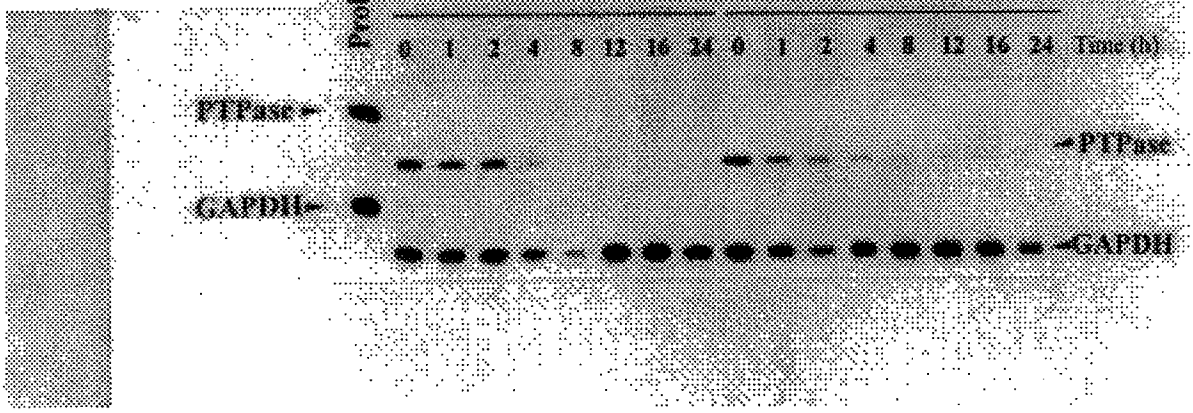


FIGURE 5A

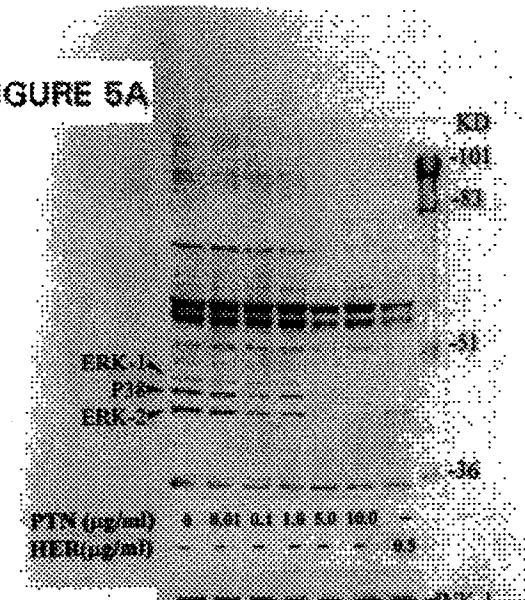
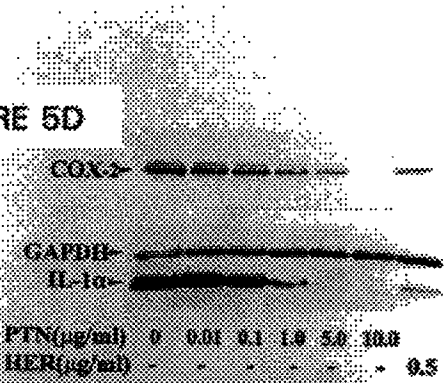


FIGURE 5B

FIGURE 5C

FIGURE 5D



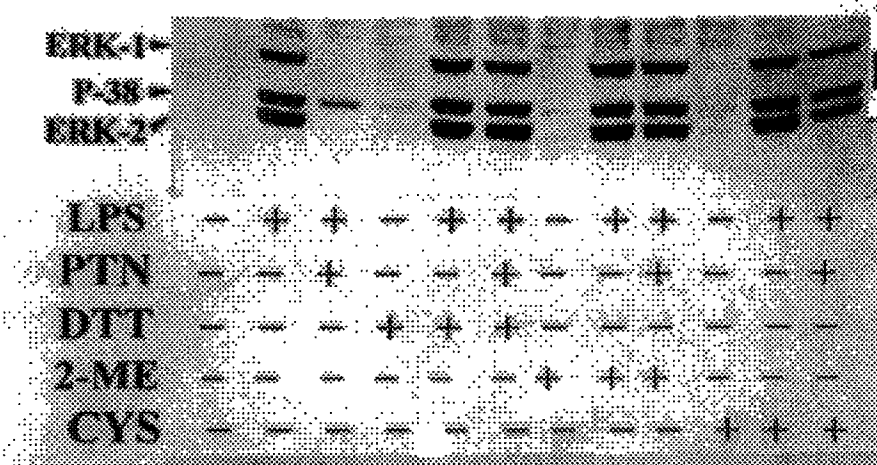


FIGURE 6A

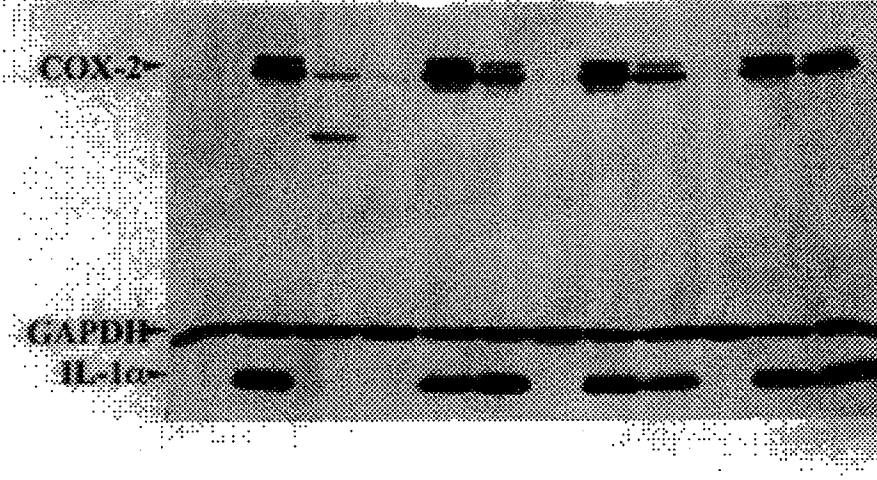


FIGURE 6B

USE OF SESQUITERPENE LACTONES FOR TREATMENT OF SEVERE INFLAMMATORY DISORDERS

The benefit of the Apr. 14, 1997 filing date of provisional application 60/080,224 (which was a conversion of nonprovisional application 08/839,514), now abandoned, is claimed under 35 U.S.C. § 119(*e*).

The development of this invention was partially funded by the Government under grant R01 DK-41868 from the National Institute of Health and grant 93-37200-8961 from the U.S. Department of Agriculture. The Government may have certain rights in this invention.

This invention pertains to sesquiterpene lactones and sesquiterpene lactone-containing plant extracts and preparations for pharmaceutical uses, particularly the use of sesquiterpene lactones for the treatment of severe inflammatory disorders, for example, sepsis, septic shock, or septicemia.

A large number of sesquiterpene lactones and their sources are described in N. Fischer et al., "The Biogenesis and Chemistry of Sesquiterpene Lactones," in W. Herz et al. (eds.), *Prog. Chem. Org. Nat. Prod.* Springer-Verlag, vol. 38, pp 47–390 (1979), the complete disclosure of which is incorporated by reference.

Sesquiterpene lactones, especially those containing an α-methylene-γ-lactone group, have been shown to possess activity against tumor growth and general inflammation. I. Hall et al., "Anti-Inflammatory Activity of Sesquiterpene Lactones and Related Compounds," *J. Pharm. Sci.,* vol. 68, pp. 537–542 (1979) discloses that sesquiterpene lactones possess activity against general inflammatory reactions. See also I. Hall et al., "Mode of Action of Sesquiterpene Lactones as Anti-Inflammatory Agents," *J. Pharm. Sci.,* vol. 69, pp. 537–543 (1980).

K. Lee, "Antitumor Agents. 32. Synthesis and Antitumor Activity of Cyclopentenone Derivatives Related to Helenalin," *J. Med. Chem.,* vol. 21, pp. 819–822 (1978) discloses that the sesquiterpene lactone helenalin and certain related compounds had some antitumor activity. See also K. Lee et al., "Cytotoxicity of Sesquiterpene Lactones," *Cancer Research,* vol. 31, pp. 1649–1654 (1971).

J. Cassady, "Potential Antitumor Agents. Synthesis, Reactivity, and Cytotoxicity of α-Methylene Carbonyl Compounds," *J. Med. Chem.,* vol. 21, pp. 815–819 (1978) reports antitumor activity of certain sesquiterpene lactones and related compounds. See also G. Howie et al., "Potential Antitumor Agents. Synthesis of Bifunctional α-Methylene-γ-butyrolactones," *J. Med. Chem.,* vol. 19, pp. 309–313 (1976).

S. Kupchan, "Tumor Inhibitors. 69. Structure-Cytotoxicity Relationships among the Sesquiterpene Lactones," *J. Med. Chem.,* vol. 14, pp. 1147–1152 (1971) discloses the structures of several cytotoxic sesquiterpene lactones, reports that an α-methylene-γ-lactone group was found to be essential for significant cytotoxic activity, and discloses other features associated with increased activity among those compounds.

T. Waddell et al., "Antitumor Agents: Structure-Activity Relationships in Tenulin Series," *J. Pharm. Sci.,* vol. 68, pp. 715–718 (1979) discloses antitumor activity of the sesquiterpene lactone tenulin and related compounds.

There are no prior reports of using a sesquiterpene lactone to treat severe inflammatory disorders such as sepsis, septic shock, or septicemia.

Septic shock and multiple-organ failure are catastrophic consequences of an invasive infection. Septic shock has been estimated to occur in more than 500,000 cases per year in the United States alone. Septic shock is the most common cause of death in non-coronary, intensive care units. As more antibiotic-resistant strains of bacteria evolve, the incidence of septic shock is expected to increase. Overall mortality rates from septic shock range from 30% to 90%. Aggressive antibiotic treatment and timely surgical intervention are the main therapies, but in many cases are insufficient. The search for new drug therapies has not been successful. R. Stone, "Search for Sepsis Drugs Goes On Despite Past Failures," *Science,* vol. 264, pp. 365–367 (1994). See, e.g., A. Fein, "Treatment of Severe Systemic Inflammatory Response Syndrome and Sepsis with a Novel Bradykinin Antagonist, Deltibant (CP-0127)," *J. Am. Med. Assoc.,* vol. 277, pp. 482–487 (1997), reporting small, but not statistically significant, improvements in 28-day mortality compared to placebo when the compound deltibant was administered to human patients suffering systemic inflammatory response syndrome and presumed sepsis. (Deltibant is a dimer of two peptides joined to one another by a linker.)

Lipopolysaccharide (LPS) is believed to be the principal agent responsible for inducing sepsis syndrome, which includes septic shock, systemic inflammatory response syndrome, and multiorgan failure. Sepsis is a morbid condition induced by a toxin, the introduction or accumulation of which is most commonly caused by infection or trauma. The initial symptoms of sepsis typically include chills, profuse sweating, irregularly remittent fever, prostration and the like; followed by persistent fever, hypotension leading to shock, neutropenia, leukopenia, disseminated intravascular coagulation, acute respiratory distress syndrome, and multiple organ failure.

LPS, also known as endotoxin, is a toxic component of the outer membrane of Gram-negative microorganisms (e.g., *Escherichia coli, Klebsiella pneumonia, Pseudomonas aeruginosa*). Compelling evidence supports the toxic role of LPS; all pathophysiological effects noted in humans during Gram-negative sepsis can be duplicated in laboratory animals by injection of purified LPS. The mechanism by which LPS activates responsive cells is complex and not fully understood. The host response to Gram-negative bacterial infection depends on effector cell recognition of the bacteria, LPS, or both, and involves both serum proteins and cell membrane receptors. When bacteria and LPS are removed via endocytosis and phagocytosis by reticuloendothial cells, concomitant activation of the host immune response by LPS results in the secretion of cytokines by activated macrophages, which in turn can trigger the exaggerated host responses associated with septic shock.

The normal immune response begins when neutrophils squeeze through the blood-vessel walls searching for bacterial pathogens in the surrounding tissue. Neutrophils can kill bacteria directly by releasing toxic chemicals or enzymes, such as elastase or collagenase. The neutrophils also attract other leukocytes to the area, including lymphocytes, macrophages, and monocytes, the last two of which release powerful immune-response activators called cytokines. The cytokines, in turn, stimulate more immune cell activity and increase the number of cells coming to the area by making the blood-vessel wall more permeable. Then, as the number of bacteria decreases, other cytokines signal to bring the normal immune response to an end.

If the cutoff mechanism fails, however, sepsis can begin. In sepsis, humoral and cellular mediators cascade in a process that becomes at least temporarily independent of the underlying infection. Excess neutrophils and macrophages are drawn to the site of infection, releasing excess immune-

stimulating cytokines, eventually triggering the release of substances that damage the blood-vessel wall. More monocytes and macrophages come to the site and release more cytokines. Eventually, the blood vessels are so damaged and leaky that blood pressure falls and the blood can no longer supply nutrients to the body's organs. Entire organs can begin to shut down. Many patients die after losing the function of two or more organs.

Two cytokines that play an important role in sepsis are interleukin-1 (IL-1) and tumor necrosis factor-alpha (TNFα). These two polypeptides can raise body temperature, increase the expression of adhesion molecules on neutrophils and endothelial cells (promoting adhesion of leukocytes), stimulate the production of vasodilating prostaglandins (thus increasing the permeability of blood vessels), trigger the release of other cytokines, stimulate neutrophils, and activate fibroblasts. All these processes enhance the probability of organ failure seen in severe septicemia. Drug therapies that targets only one of these two cytokines have proved ineffective. See Stone (1994). Drug therapies that are effective against general inflammatory responses have not proven to be effective against the cascading acute inflammation that produces septicemia. There is a need for drugs that can inhibit this cascading system at the beginning steps of production of IL-1 and TNFα.

Other important cytokines, chemokines, and other proteins having proinflammatory activity include interferon-gamma (IFN-γ), interleukin-6 (IL-6), macrophage chemotactic protein (MCP), inducible nitric oxide synthetase (iNOS), mitogen-activated protein kinases (MAPKs), macrophage inflammatory protein, KC/CINC (growth related gene), tissue factor (TF), granulocyte-macrophage-colony stimulating factor (GM-CSF) and phosphotyrosine phosphatase (PTPase).

Prostaglandins are also involved in the proinflammatory response; e.g., prostaglandins increase the permeability of the blood-vessel wall. Cyclooxygenase (COX; prostaglandin endoperoxide synthase) catalyzes the conversion of arachidonic acid to prostaglandin (PG) endoperoxide (PGH2), which is the rate limiting step in prostaglandin biosynthesis. Two isoforms of COX have been cloned from animal cells: the constitutively expressed COX-1, and the mitogen-inducible COX-2. Prostaglandins produced as a result of the activation of COX-1 may have physiological functions such as the antithrombogenic action of prostacyclin released by the vascular endothelium, and the cytoprotective effect of PGs produced by the gastric mucosa. However, COX-2 is the enzyme expressed following the activation of cells by various proinflammatory agents including cytokines, endotoxin and other mitogens. These observations suggest that COX-2 instead of COX-1 may be responsible for inducing production of the prostaglandins involved in inflammation. Only a few pharmacological agents that suppress the expression of COX-2 without affecting COX-1 have not been identified, for example, glucocorticoids and radicicol.

There is a need for compounds that selectively inhibit COX-2, and that act as potent anti-inflammatory agents, with minimal side effects. To prevent septicemia, such a compound should also inhibit the production of a wide variety of proinflammatory cytokines, especially TNFα and IL-1, chemokines, and protein-tyrosine kinases.

Protein-tyrosine kinases (PTK) play a key role in initiating both receptor-mediated and non-receptor-mediated signal transduction pathways in eukaryotic cells. Increased PTK activity has been associated with cancers and with acute inflammatory responses such as septic shock. Lipopolysaccharide (LPS) antigen activates macrophages, monocytes, and neutrophils, and the activated cells produce proinflammatory cytokines and lipid mediators that initiate and amplify inflammatory responses. LPS also stimulates protein tyrosine phosphorylation of mitogen-activated protein kinases (MAPKs) in macrophages. Suppression of the LPS-induced tyrosine phosphorylation results in inhibition of the expression of proinflammatory cytokines and of COX-2. We have shown that the activation of MAPKs is required for the expression of COX-2 (our laboratory, unpublished data).

P. Chanmugam, "Radicicol, a Protein Tyrosine Kinase Inhibitor, Suppresses the Expression of Mitogen-Inducible Cyclooxygenase in Macrophages Stimulated with Lipopolysaccharide and in Experimental Glomerulonephritis," *J. Biol. Chem.*, vol. 270, pp. 5418–5426 (1995) discloses that radicicol, a protein-tyrosine kinase inhibitor, suppresses the expression of mitogen-inducible cyclooxygenase 2 in macrophages stimulated with lipopolysaccharide. See also co-pending patent application Ser. No. 08/394,148, filed Feb. 24, 1995, which is assigned in part to the assignee of the present application.

Inhibitors of protein-tyrosine kinases have been shown to be effective in decreasing mortality in LPS-induced septicemia. A. Novogrodsky et al., "Prevention of Lipopolysaccharide-Induced Lethal Toxicity by Tyrosine Kinase Inhibitors," *Science*, vol. 264, pp. 1319–1322 (1994) discloses that protein tyrosine kinase inhibitors of the tyrphostin AG126 family protected mice against lipopolysaccharide-induced lethal toxicity.

Thus lipopolysaccharide (LPS) stimulates protein tyrosine phosphorylation in macrophages and induces the expression of the mitogen-inducible cyclooxygenase COX-2 and TNFα. PTK inhibitors suppress the expression of cyclooxygenase and TNFα in macrophages. The synthetic PTK inhibitors, tyrphostins, have been shown to prevent LPS-induced lethal toxicity in mice. These results indicate that PTK inhibitors may be effective therapeutic agents for septic shock and other acute inflammatory disorders.

The PTK inhibitors reported have been derived from the class of natural products called flavonoids, e.g., quercetin, genistein, levendustin A, erbstatin and herbimycin A. T. Akiyama et al., "Genistein, a Specific Inhibitor of Tyrosine-Specific Protein Kinases," *Journal of Biological Chemistry*, vol. 262, pp. 5592–5595 (1986) discloses that genistein, an isoflavone, inhibited the tyrosine-specific protein kinase activity of the epidermal growth factor receptor in vitro. Synthetic PTK inhibitors include tyrphostins, which contain the benzylidene moiety of erbstatin and other arylidene compounds, and a specific inhibitor of the epidermal growth factor receptor tyrosine kinase. There is no prior report of a sesquiterpene lactone inhibiting a protein-tyrosine kinase.

U.S. Pat. No. 4,758,433 discloses that sesquiterpene lactones derived from *Tanacetum parthenium* are useful in treating migraine, and that they may also be useful in treating asthma and arthritis.

U.S. Pat. No. 5,384,121 discloses a method for extracting sesquiterpene lactones from *Tanacetum parthenium*.

We have discovered that sesquiterpene lactones possessing an α-methylene γ-lactone group suppress the expression of the inducible cyclooxygenase-2, and also suppress the proinflammatory cytokines interleukin-1α, interleukin-1β, IL-1, and tumor necrosis factor-α(TNFα), and chemokines in mammalian macrophages stimulated with lipopolysaccharide. These sesquiterpene lactones also inhibit protein-tyrosine phosphorylation, including the mitogen-activated protein kinases. Sesquiterpene lactones are useful in sup-

pressing early proinflammatory cytokines and proteins, and in ameliorating severe inflammatory disorders, including sepsis, septic shock, and septicemia.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 illustrates the chemical structures of representative sesquiterpene lactones.

FIG. 2 illustrates observed dose-response for parthenolide inhibition of de novo synthesis of COX-2 and TNFα in LPS-stimulated macrophages.

FIG. 3A illustrates the dose-dependent inhibition of the expression of COX-2 and IL-1α proteins by parthenolide.

FIG. 3B illustrates the dose-dependent inhibition of the steady state levels of COX-2 and GAPDH mRNAs by parthenolide.

FIGS. 4A and 4B illustrate the time course for steady state levels of mRNA for TNFα, IL-1β, PTPase and GAPDH.

FIG. 5A illustrates the dose-dependent inhibition of protein tyrosine phosphorylation and kinase activity of extracellular signal-regulated protein kinase-1 (ERK-1) and -2 (ERK-2) by parthenolide and herbimycin A as measured by antiphosphotyrosine immunoblot.

FIG. 5B illustrates the dose-dependent inhibition of protein tyrosine phosphorylation and kinase activity of ERK-1 and ERK-2 by parthenolide and herbimycin A as measured by c-Jun N-terminal kinase-1 (JNK-1) immunoblot.

FIG. 5C illustrates the dose-dependent inhibition of protein tyrosine phosphorylation and kinase activity of ERK-1 and ERK-2 as measured by an in-gel kinase assay using myelin basic protein (MBP) as a substrate.

FIG. 5D illustrates the dose-dependent inhibition of protein tyrosine phosphorylation and kinase activity of ERK-1 and ERK-2 as measured by COX-2, IL-1α, and GAPDH immunoblots.

FIGS. 6A and 6B illustrate the suppression of the inhibitory effects of parthenolide on tyrosine phosphorylation of MAPKs and expression of COX-2 and IL-1α by certain sulfhydryl compounds.

The present invention provides a new use for the class of compounds known as sesquiterpene lactones, especially the subclass containing an α-methylene-γ-lactone moiety. Representative sesquiterpene lactones have been observed to inhibit IL-1, TNFα, and protein tyrosine kinases.

In a preferred embodiment, the present invention provides a method of treating a severe inflammatory disorder in a mammal, including a human, comprising administering to the mammal with the disorder a therapeutically effective amount of a sesquiterpene lactone having an α-methylene-γ-lactone group.

Sesquiterpenes are terpene compounds with fifteen carbon atoms; the biogenesis of a naturally-occurring sesquiterpenes is derivation from three mevalonic acid molecules as starting materials. Sesquiterpene lactones are a subclass of sesquiterpenes having a lactone functionality; a lactone is a cyclic ester. Many sesquiterpene lactones contain an exocyclic methylene lactone group, the α-methylene-γ-lactone moiety. See N. Fischer et al. (1979). Sesquiterpene lactones with the α-methylene-γ-lactone moiety show increased bioactivity if at least one of the following additional alkylating functional groups is present: an epoxide, a cyclopentenone, a cyclohexenone, a cyclohexadienone, an α,β-unsaturated ester, an α,β-epoxy ester, an α,β-unsaturated aldehyde, or an α, β-unsaturated ketone.

FIG. 1 illustrates the structures of representative sesquiterpene lactones. Compounds (1)–(10) contain an α-methylene-γ-lactone moiety. Compound (1), parthenolide, contains an epoxide moiety. Compound (2), encelin, contains a cyclohexadienone moiety. Compounds (3)–(5), leucanthin B, enhydrin, and melampodin, respectively, contain an α,β-unsaturated ester and α,β-expoxy ester side chains. Compounds (6)–(9) contain only the α-methylene-γ-lactone moiety. Compound (10) contains a cyclopentenone group. Compounds (11)–(12), 11,13-dihydroparthenolide and 1,10-epoxy-11,13-dihydroparthenolide, respectively, are derivatives of parthenolide that have lost the α-methylene-γ-lactone moiety. Compound (13), santonin, contains the epoxide moiety, but does not contain the α-methylene-γ-lactone moiety. Only the first ten compounds were found to be active.

As used herein, the term "active sesquiterpene lactone" refers to a sesquiterpene lactone that has an α-methylene-γ-lactone functional group, and that is capable of inhibiting or reducing the severity of a severe inflammatory response. Such active sesquiterpene lactones may be used in various combinations or mixtures.

An active sesquiterpene lactone may be administered to a patient by any suitable means, including parenteral, subcutaneous, intrapulmonary, and intranasal administration. Parenteral infusions include intramuscular, intravenous, intraarterial, or intraperitoneal administration. Active sesquiterpene lactone may also be administered transdermally, for example in the form of a slow-release subcutaneous implant, or orally in the form of capsules, powders, or granules. They may also be administered by inhalation.

Pharmaceutically acceptable carrier preparations for parenteral administration include sterile, aqueous or non-aqueous solutions, suspensions, and emulsions. Examples of non-aqueous solvents are propylene glycol, polyethylene glycol, vegetable oils such as olive oil, and injectable organic esters such as ethyl oleate. Aqueous carriers include water, alcoholic/aqueous solutions, emulsions or suspensions, including saline and buffered media. Parenteral vehicles include sodium chloride solution, Ringer's dextrose, dextrose and sodium chloride, lactated Ringer's, or fixed oils. The active therapeutic ingredient may be mixed with excipients that are pharmaceutically acceptable and are compatible with the active ingredient. Suitable excipients include water, saline, dextrose, glycerol and ethanol, or combinations thereof. Intravenous vehicles include fluid and nutrient replenishers, electrolyte replenishers, such as those based on Ringer's dextrose, and the like. Preservatives and other additives may also be present such as, for example, antimicrobials, anti-oxidants, chelating agents, inert gases, and the like.

The form may vary depending upon the route of administration. For example, compositions for injection may be provided in the form of an ampule, each containing a unit dose amount, or in the form of a container containing multiple doses.

Active sesquiterpene lactone may be formulated into therapeutic compositions as pharmaceutically acceptable salts. These salts include the acid addition salts formed with inorganic acids such as, for example, hydrochloric or phosphoric acid, or organic acids such as acetic, oxalic, or tartaric acid, and the like. Salts also include those formed from inorganic bases such as, for example, sodium, potassium, ammonium, calcium or ferric hydroxides, and organic bases such as isopropylamine, trimethylamine, histidine, procaine and the like.

Controlled delivery may be achieved by admixing the active ingredient with appropriate macromolecules, for

example, polyesters, polyamino acids, polyvinyl pyrrolidone, ethylenevinylacetate, methylcellulose, carboxymethylcellulose, prolamine sulfate, or lactide/glycolide copolymers. The rate of release of the active sesquiterpene lactone may be controlled by altering the concentration of the macromolecule.

Another method for controlling the duration of action comprises incorporating the active sesquiterpene lactone into particles of a polymeric substance such as a polyester, peptide, hydrogel, polylactide/glycolide copolymer, or ethylenevinylacetate copolymers. Alternatively, an active sesquiterpene lactone may be encapsulated in microcapsules prepared, for example, by coacervation techniques or by interfacial polymerization, for example, by the use of hydroxymethylcellulose or gelatin-microcapsules or poly (methylmethacrylate) microcapsules, respectively, or in a colloid drug delivery system. Colloidal dispersion systems include macromolecule complexes, nanocapsules, microspheres, beads, and lipid-based systems including oil-in-water emulsions, micelles, mixed micelles, and liposomes.

A severe inflammatory disorder treated by the method of the present invention may be associated with expression of COX-2 or proinflammatory agents such as cytokines, TNF, IL-1, and MAPKS production, for example.

The present invention provides a method of treating or ameliorating a severe inflammatory disorder such as sepsis, endotoxemia, or septic shock, or one or more of the symptoms of sepsis; comprising administering to a subject displaying such symptoms or at risk for developing sepsis, a therapeutically effective amount of an active sesquiterpene lactone. The term "ameliorate" refers to a decrease or lessening of the symptoms of the disorder being treated. The symptoms that may be ameliorated include those associated with a transient increase in the blood level of TNF, such as fever, hypotension, neutropenia, leukopenia, thrombocytopenia, disseminated intravascular coagulation, adult respiratory distress syndrome, shock, and multiple organ failure. Patients who may benefit from such treatment include those at risk for or those suffering from toxemia, such as endotoxemia resulting from a Gram-negative bacterial infection, venom poisoning, or hepatic failure, for example. In addition, patients having a Gram-positive bacterial, viral, or fungal infection may also display symptoms of sepsis, and may also benefit from the therapeutic method described here.

Patients likely to benefit from the method of the present invention include those suffering from infection by Gram negative bacteria such as *E. coli, Haemophilus influenza B, Neisseria meningitides,* staphylococci, or pneumococci. Patients at risk for developing sepsis include those suffering from burns, gunshot wounds, renal failure, hepatic failure, trauma, burns, immunodepression (including HIV infection), hematopoietic neoplasias, multiple myeloma, Castleman's disease, or cardiac myxoma.

The term "therapeutically effective amount" as used herein for treatment of septicemia or endotoxemia refers to an amount of an active sesquiterpene lactone sufficient to decrease the subject's response to LPS, or to decrease the symptoms of sepsis or other severe inflammatory disorder. The term "therapeutically effective amount" therefore includes, for example, an amount of an active sesquiterpene lactone sufficient to prevent, and preferably to reduce by at least 50%, and more preferably sufficient to reduce by at least 90%, a clinically significant increase in a patient's plasma level of TNFα. The dosage ranges for the administration of active sesquiterpene lactone are those that produce the desired effect. Generally, the dosage will vary with the age, condition, and sex of the patient, and the extent of the infection. A person of ordinary skill in the art, given the teachings of the present specification, may readily determine suitable dosage ranges. The dosage can be adjusted by the individual physician in the event of any contraindications. In any event, the effectiveness of treatment can be determined by monitoring the level of LPS, IL-1, and TNF in a patient. A decrease in serum LPS and TNF levels should correlate with recovery of the patient.

In addition, patients at risk for or exhibiting the symptoms of sepsis can be treated by the novel method, substantially simultaneous with the therapeutic administration of another TNF inhibitor. For example, administering an anti-TNF antibody or a TNF antagonist can help prevent or ameliorate the symptoms of sepsis. Particularly preferred is the use of an anti-TNF antibody as an active ingredient, such as a monoclonal antibody with TNF specificity as described by Tracey, et al. *Nature,* vol. 330, p. 662 (1987).

A patient who exhibits the symptoms of sepsis may also be treated with an antibiotic in addition to the treatment with active sesquiterpene lactone. Typical antibiotics include an amino-glycoside, such as gentamycin or a beta-lactam such as penicillin or cephalosporin. Therefore, a preferred therapeutic method includes administering a therapeutically effective amount of an active sesquiterpene lactone substantially simultaneously with administration of a bactericidal amount of an antibiotic. Preferably, administration of active sesquiterpene lactone occurs within about 48 hours and preferably within about 2–8 hours, and most preferably, substantially concurrently with administration of the antibiotic.

The term "bactericidal amount" refers to an amount sufficient to achieve a bacteria-killing blood concentration in the patient receiving the treatment. The bactericidal amounts of antibiotics generally recognized as safe for administration to a human are well known in the art, and as is known in the art, vary with the specific antibiotic and the type of bacterial infection being treated.

Administration of an active sesquiterpene lactone may also be used for ameliorating post-reperfusion injury. When treating arterial thrombosis, induction of reperfusion by clot lysing agents such as tissue plasminogen activator (t-PA) is often associated with tissue damage. Such tissue damage is thought to be mediated at least in part by leukocytes, including polymorphonuclear leukocytes (PMN). Administration of an active sesquiterpene lactone blocks leukocyte or PMN-endothelial interactions, and thereby diminish or prevent post-reperfusion injury.

The method is also useful in treating non-malignant or immunologically-related cell proliferative diseases such as psoriasis, pemphigus vulgaris, Behcet's syndrome, acute respiratory distress syndrome (ARDS), ischemic heart disease, post-dialysis syndrome, leukemia, acquired immune deficiency syndrome, septic shock, other types of acute inflammation, and lipid histiocytosis. Any disorder that is etiologically linked to the proinflammatory process (e.g., induction of IL-1, TNF-α, COX-2 expression) may be treated by this method.

While not wishing to be bound by a particular theory, it is believed that an active sesquiterpene lactone suppresses tyrosine phosphorylation of protein-tyrosine kinases, and that inhibition of COX-2 expression by an active sesquiterpene lactone is mediated at least in part by the inhibition of protein-tyrosine kinases.

The effectiveness of treatment may be monitored by detection methods used in the art, including immunoassays, Northern and Western blot analysis, and RNase protection assays. Examples of immunoassays that may be used to detect and monitor levels of cytokines, chemokines, mitogens, or other proteins affected by an active sesquiterpene lactone in a sample include competitive and non-competitive immunoassays, in either a direct or indirect format, such as a radioimmunoassay (RIA) or a sandwich (immunometric) assay. An immunoassay of a protein may be run in forward mode, reverse mode, or simultaneous modes, including competition immunoassays, and immunohistochemical assays on physiological samples. Monitoring is preferably performed by a forward immunoassay. Those of skill in the art will know, or can readily discern, other immunoassay monitoring formats without undue experimentation.

Solid phase-bound antibody molecules can be bound by adsorption from an aqueous medium, although other modes of fixation, such as covalent coupling or other known means of fixation to a solid matrix may be used. Preferably, the first antibody molecule is bound to a support before forming an immunocomplex with antigen (e.g., cytokine); however, the immunocomplex may also be formed prior to binding the complex to the solid support.

Non-specific protein binding sites on the surface of the solid phase support are preferably blocked. After adsorption of solid phase-bound antibodies, an aqueous solution of a protein free from interference with the assay—such as bovine, horse, or other serum albumin—that is also free from contamination with the antigen, is admixed with the solid phase to adsorb the admixed protein onto the surface of the antibody-containing solid support at protein binding sites on the surface that are not occupied by the antibody molecule.

A typical aqueous protein solution contains about 2–10 weight percent bovine serum albumin in phosphate-buffered saline (PBS) at a pH about 7–8. The aqueous protein solution-solid support mixture is typically maintained for a time period of at least one hour at a temperature of about 37–40° C., and the resulting solid phase is thereafter rinsed free of unbound protein.

The first antibody can be bound to different carriers and used to detect a cytokine or other protein in a sample. Examples of such carriers include glass, polystyrene, polypropylene, polyethylene, dextran, nylon, amyloses, natural and modified celluloses, polyacrylamides, agaroses, and magnetite. The carrier may be soluble or insoluble. Those skilled in the art will know of other suitable carriers for binding antibodies or antigen, or will be able to ascertain such carriers through routine experimentation.

In addition, if desired, an antibody in these immunoassays can be detectably labeled in various ways. There are many different labels and methods of labeling known to those skilled in the art. Examples of the types of labels which can be used in the present invention include enzymes, radioisotopes, fluorescent compounds, colloidal metals, chemiluminescent compounds, and bioluminescent compounds. Those of skill in the art will known of other suitable labels for binding to monoclonal antibodies, or will be able to ascertain such labels through routine experimentation. Furthermore, binding such labels to the antibodies may be performed with routine methods known in the art.

One of skill in the art may monitor the effect of an active sesquiterpene lactone on a protein kinase by measuring changes in the level of kinase activity. Such a measurement may comprise incubating the components, which include the kinase or a polynucleotide encoding the kinase and its substrate (e.g., Src tyrosine kinase and p53/56^{lyn}), under conditions conducive to interaction of the components, and then measuring the effect the composition has on kinase activity. For example, an increase or decrease in kinase activity may be measured by adding a radioactive compound to the mixture of components, such as ^{32}P-ATP, and observing incorporation of radioactivity into the substrate to assay the compound's effect on protein kinase activity. A polynucleotide encoding the kinase may be inserted into an expression vector, and the effect of a composition on transcription of the kinase or stability of the mRNA may be measured, for example, by Northern blot analysis or RNase protection assay (see for example, Current Protocols in Molecular Biology, Ausubel, et al., *Wiley Interscience,* 1994, incorporated herein by reference). The level of cytokine, chemokine, mitogen, or other protein inhibited by an active sesquiterpene lactone may also be monitored by these and other standard techniques known to those of skill in the art.

The following examples are intended to illustrate but not limit the invention. While they are typical of those that might be used, other procedures otherwise known to those skilled in the art may alternatively be used.

EXAMPLE 1: Materials and Methods

1. Preparation of Parthenolide and Other Sesquiterpene Lactones

Parthenolide (compound 1 in FIG. 1) was extracted from dried leaves of *Magnolia grandiflora* as described originally by El-Feraly et al., *J. Pharma. Sci.,* vol. 67, 347–350 (1978). Encelin (compound 2 in FIG. 1) was extracted from *Encelia farinosa* as described by Geissman et al., *J. Org. Chem.,* vol. 33, p. 656 (1978). Leucanthin B and Melampodin A (Compounds 3 and 5, respectively, in FIG. 1) were extracted from *Melampodium leucanthum* as described by Fischer et al., *Phytochemistry,* vol. 14, p. 2241 (1975). Enhydrin (Compound 4 in FIG. 1) was extracted from *Polymnia uvedalia* as described by N. Fischer, *Rev. Latinoamer. Quim.,* vol. 9, p. 41 (1978). Confertiflorin (Compound 6 in FIG. 1) was extracted from *Ambrosia confertiflora* as described by Fischer et al., *Tetrahedron,* vol. 23, p. 2529 (1967). Burrodin (Compound 7 in FIG. 1) was extracted from *Ambrosia dumosa* as described by Geissman et al., *Phytochemistry,* vol. 7, p. 1613 (1968). Psilostachyin A (Compound 8 in FIG. 1) was extracted from *Ambrosia artemisiifolia* as described by Herz et al., *Phytochemistry,* vol. 12, p. 1415 (1975). Costunolide (Compound 9 in FIG. 1) was extracted from *Saussurea lappa* (Costus root oil) as described by Rao et al., *Tetrahedron,* vol. 9, p. 275 (1960). Tenulin (Compound 10 in FIG. 1) was extracted from *Helenium amarum* as described by Herz et al., *J. Amer. Chem. Soc.,* vol. 84, p. 3857 (1962). Compound 11, 11,13-dihydroparthenolide (FIG. 1), was extracted from *Ambrosia artemisiifolia.* Compound 12, 1(10)epoxy-11,13-dihydroparthenolide (FIG. 1), is a synthetic derivative of Compound 11. Santonin (Compound 13 in FIG. 1) is commercially available from Aldrich. The structural identities of parthenolide and other sesquiterpene lactones were determined spectroscopically (^{1}H and ^{13}C NMR, IR, MS) as described in Fischer et al. (1979).

2. Isolation of Macrophages

Rat (Sprague-Dawley) alveolar macrophages were collected by broncho-alveolar lavage as described by Lee et al.,

J. Biol. Chem., vol. 267, pp. 25934–25938 (1992). The murine macrophage cell line RAW 264.7 (ATCC no. TIB-71) was cultured in Dubecco's modified Eagle's medium (DMEM) containing 10% heat-inactivated fetal bovine serum (FBS, Intergen). For the cyclooxygenase activity assay, cells were seeded in 24 well plates, and after near-confluency, cells were treated with aspirin (250 μM) for 2.5h to inactivate endogenous cyclooxygenase. The time course for the COX activity indicated that the maximum increase was reached in 8h. COX activity was determined by measuring prostaglandin E_2 concentrations in cells incubated with arachidonic acid (30 μM) for 10 minutes as described in Lee et al. (1992).

3. Antiphosphotyrosine Immunoblotting

This detection technique was carried out essentially as described in Chanmugan et al. (1995) using 4G10 monoclonal antiphosphotyrosine antibody (UBI) and the ECL detection system (Amersham).

4. Western Blot Analyses for COX-2, Interleukin-1α (IL-1α), c-Jun N-terminal Kinase-1 (JNK-1) and Glyceraldehyde-3-phosphate Dehydrogenase (GAPDH) Proteins

The protein levels of COX-2 and GAPDH were assessed by Western blot analysis using polyclonal antibodies as described in Lee et al. (1992). Polyclonal antibodies for IL-1α and JNK-1 were purchased from Genzyme and Santa Cruz Biotech, respectively.

5. RNase Protection Assay

Total cellular RNA was isolated by TRIzoL reagent (Gibco, BRL). The RNase protection assay was performed as described in Chanmugan et al. (1995).

6. In-gel Kinase Assay

This assay was performed according to the method described by Kameshita et al., *Anal. Biochem.*, vol. 183, pp. 139–143 (1989) using myelin basic protein (MBP) as a substrate.

EXAMPLE 2: Inhibition of the Expression of Cyclooxygenase and Proinflammatory Cytokines by Parthenolide in LPS-Stimulated Alveolar Macrophages

Recovered COX activity in cells pretreated with aspirin reflected de novo synthesized COX-2. Alveolar macrophages pretreated with aspirin were incubated with LPS (10 μg/ml) and various concentrations of parthenolide for 16h. The activity of de novo synthesized COX-2 was determined by measuring the levels of PGE_2 produced from exogenous arachidonic acid. Activity of TNFα was determined by bioassay using L929 cells as described by Aggarwal et al., *J. Biol. Chem.*, vol. 260, pp. 2345–2354 (1985). The dose-response to parthenolide in inhibiting the expression of COX activity showed that the IC_{50} was about 0.8 μM as shown in FIG. 2. Values in FIG. 2 are the means of triplicate samples for TNFα and duplicate samples for PGE_2.

Similar inhibitions of the expression of COX-2 protein and steady state levels of COX-2 mRNA are shown in FIG. 3. In FIG. 3A, alveolar macrophages were incubated with LPS and various concentrations of parthenolide for 16h. Solubilized proteins were analyzed by COX-2, IL-1α or GAPDH immunoblotting. FIG. 3A is a representative immunoblot of more than five different analyses. Data are shown for various concentrations of both parthenolide (PTN) and herbimycin A (HER). Whether the suppression of the steady state levels of COX-2 mRNA by parthenolide was due to inhibition of the transcription rate or to accelerated degradation of mRNA is not known.

Parthenolide suppressed LPS-induced TNFα production with an IC_{50} of 0.1 μg/ml, as seen in FIG. 2. Steady state levels of mRNA for TNFα and IL-1β were also inhibited by parthenolide as shown in FIG. 3B and FIG. 4. In FIG. 3B, alveolar macrophages were incubated with LPS in the presence of various concentrations of parthenolide for 2h. Steady state levels of mRNA were determined by RNase protection assay. In FIG. 4, alveolar macrophages were incubated with LPS in presence of 1 μg/ml of parthenolide for specified time periods. The concentrations of mRNA were determined by RNase protection assay.

Parthenolide inhibited the expression of IL-1α protein (non-secreted precursor form of IL-1) as determined by Western blot analysis as seen in FIG. 3A.

EXAMPLE 3. Parthenolide Suppresses Tyrosine Phosphorylation of Proteins, Including the Mitogen-Activated Protein Kinases (MAPKs)

The stimulation of macrophages by LPS results in the activation of MAPKs that lie at a central point in the multiple signal transduction pathways for various growth factors, hormones, and cytokines. Extracellular signal-regulated protein kinase 1 and 2 (ERK1 and ERK2) require phosphorylation of both Thr-183 and Tyr-185 for activation.

Parthenolide suppressed LPS-stimulated tyrosine phosphorylation of various proteins in RAW 264.7 cells as assessed by antiphosphotyrosine immunoblot, depicted in FIG. 5A. This inhibition was correlated with the suppressed expression of COX-2 and IL-1α. RAW 264.7 cells were pretreated with parthenolide in various concentrations or with herbimycin A (0.5 μg/ml) for 3h, and then stimulated with LPS (1 μg/ml) in the presence of the inhibitors for 30 min. FIG. 5 shows the dose-dependent inhibition of protein tyrosine phosphorylation and kinase activity of ERK-1 and ERK-2 by parthenolide and herbimycin A. FIG. 5A shows activity as measured by an antiphosphotyrosine immunoblot. The figure shows a representative immunoblot from more than five different analyses. Among these proteins, MAPKs exhibited the most dramatic inhibition in the extent of tyrosine phosphorylation in response to parthenolide. Parthenolide inhibition of tyrosine phosphorylation was most strongly inhibited in MAPKs. The tyrosine phosphorylation of three MAPK subfamily enzymes (ERK-1, ERK-2 and P38), which are all stimulated by LPS, was inhibited by parthenolide in a dose-dependent manner (FIG. 5A). The monoclonal antiphosphotyrosine antibody (4G10) did not recognize phosphorylated c-Jun N-terminal kinase-1 (JNK-1). Therefore, the extent of tyrosine phosphorylation of JNK-1 was assessed by the electrophoretic mobility shift of phosphorylated JNK-1 as shown in FIG. 5B. Parthenolide inhibited tyrosine phosphorylation of JNK-1. Another protein tyrosine kinase inhibitor, herbimycin A (a natural flavanoid), inhibited tyrosine phosphorylation of the MAPK subfamily.

The inhibition of tyrosine phosphorylation of MAPKs correlated with the inhibition of COX-2 and IL-1α expression in RAW 264.7 cells as shown in FIG. 5D.

The mechanism by which parthenolide inhibits protein tyrosine phosphorylation is not currently known. It has been speculated that the tyrosine kinase inhibitor herbimycin A inactivates $p60^{v\text{-}src}$ kinase by irreversibly binding to the

sulphydryl (SH) groups of $p60^{v\text{-}src}$ kinase. Y. Uehara et al., *Biochem. Biophys. Res. Commun.*, vol. 163, pp. 803–809 (1989). The inactivation by herbimycin may occur through conjugation between highly polarized double bonds in the benzoquinone moiety of herbimycin A and the SH group of sulphydryl compounds. Without wishing to be bound by this theory, the α-methylenebutyrolactone in parthenolide may interact with biological nucleophiles such as sulphydryl groups. Indeed, pretreating cells with sulphydryl compounds abrogated the inhibitory effect of parthenolide on LPS-induced activation of MAPKs as shown in FIG. 6A. In FIG. 6, RAW 264.7 cells were pretreated with parthenolide (1 μg/ml) in the presence of DTT (dithiothreitol, 100 μM), 2-ME (2-mercaptoethanol, 50 μM), or Cys (L-cysteine, 150 μM), and were then stimulated with LPS (1 μg/ml). Cells were incubated for 30 min for antiphosphotyrosine immunoblot analysis (FIG. 6A) and 8h for COX-2 and IL-1α Western blot analyses (FIG. 6B). Abolishing the inhibitory effect of parthenolide on the activation of MAPKs by these agents resulted in recovered expression of COX-2 and IL-1α that had been inhibited by parthenolide (FIG. 6B). These results imply that the inhibitory effects of parthenolide are mediated through conjugation with SH-groups of target proteins. However, these data alone do not permit an identification of the specific target protein(s) affected by parthenolide. Whether parthenolide inhibits protein tyrosine phosphorylation by directly inhibiting PTKs or by inhibiting other target protein(s) that affect the activity of PTKs is not currently known.

EXAMPLE 4. Structure—Function Relationships Among Sesquiterpene Lactones in Inhibiting COX-2 Expression

Among the sesquiterpene lactones tested to date, parthenolide, encelin, and leucanthin B (Compounds 1, 2, and 3, respectively in Table 1 and FIG. 1) have shown the highest inhibitory activity.

TABLE 1

Relative potencies of different sesquiterpene lactones in inhibiting the expression of COX-2 in LPS-stimulated macrophages[a]

Compound No. in FIG. 1	Common Name	Mol. Wt.	IC_{50} (μ/ml)
1	Parthenolide	248	0.2
2	Encelin	244	0.1
3	Leucanthin B	478	0.2
4	Enhydrin	464	0.3
5	Melampodin A	444	0.5
6	Confertiflorin	306	0.9
7	Burrodin	264	1.0
8	Psilostachyin A	280	1.3
9	Costunolide	232	1.6
10	Tenulin	306	5.5
11	11,13-Dihydroparthenolide	250	>100
12	1(10)Epoxy-11,13-Dihydroparthenolide	266	>100
13	Santonin	246	>100

[a]IC_{50} was determined at multiple dose levels.

A common feature of the compounds with strong inhibitory activity was that they each possess an α-methylene-γ-lactone functional group, as well as another conjugation site. Compound 1, parthenolide, contains an epoxide moiety. Compound 2, encelin, in addition to the α-methylene-γ-lactone moiety possesses a cyclohexadienone structure, thus giving it three possible conjugation sites. Encelin was the most active compound tested. Compounds 3, 4 and 5 are examples of sesquiterpene lactones bearing epoxides(s) with α,β-unsaturated ester and α,β-epoxy ester side chains; these compounds exhibited activities similar to that of parthenolide.

Compounds 6 through 9 contain only the α-methylene-γ-lactone moiety, and their inhibitory activity was lower than that of compounds 1–5. Compound 10 is an example of a cyclopentenone; its activity was significantly less than that of compounds 1–9. Compounds 11 and 12, containing an epoxide moiety but no α-methylene-γ-lactone group, showed loss of inhibitory activity. Although the presence of an epoxide functionality appeared to accentuate the inhibitory activity of sesquiterpene lactones containing an α-methylene-γ-lactone group, an epoxide alone did not confer inhibitory activity to a sesquiterpene.

While not limiting the scope of the invention, examples of other active sesquiterpene lactones that may prove especially effective include ambrosin, aromaticin, burrodin, cinerenin, costunolide, chammissonin, coronopilin, confertiflorin, encelin, enhydrin, elephantopin, elephantin, eupatrindin, eupachlorin, euparotin, eupacunin, elephantol, eupahyssopin, eupatolide, eupaformosanin, farinosin, vernolepin, vernomenin, xanthanin, gaillardin, helenalin, leucanthin, ludovicin, liatrin, melampodin A and B, molephantin, molephantinin, mexicanin, parthenolide, paucin, parthenin, psilostachyin, tenulin, and tamaulipin.

EXAMPLE 5: Inhibition of Nuclear Factor—kB

Parthenolide inhibits Nuclear Factor—kB (NF-kB) transcription factor that has been activated by LPS in the murine macrophage cell line (RAW 264.7) as assessed by the degradation of the protein, IKB_{α}. Activated NF-kB is known to induce the expression of many early response genes including inducible nitric oxide synthetase, cyclooxygenase, and chemokines that are implicated in acute inflammatory responses. Without wishing to be bound by this theory, this response may provide a part of the mechanism by which parthenolide inhibits the expression of COX-2, and further suggests that parthenolide also suppresses the production of nitric oxide and other proinflammatory cytokines.

EXAMPLE 6: In Vivo Experiments in Mice

Varying doses of parthenolide will be injected intraperitoneally into mice along with a lethal dose of LPS (1.5 mg/mouse). The mortality of the mice will be monitored for one week. Blood levels of TNFα will be monitored as described by Novogrodsky et al. (1994).

Once satisfactory data from laboratory animals have been obtained, clinical trials in human patients will be conducted in accordance with applicable statutes and regulations.

The complete disclosures of all references cited in this specification are hereby incorporated by reference. In the event of an otherwise irreconcilable conflict, however, the present specification shall control. Also incorporated by reference is the complete disclosure of the following paper, which is not prior art to the present invention: D. Hwang et al., "Inhibition of the Expression of Inducible Cyclooxygenase and Proinflammatory Cytokines by Sesquiterpene Lactones in Macrophages Correlates with the Inhibition of MAP Kinases," *Biochem. and Biophys. Res. Comm.*, vol. 226, pp. 810–818 (1996).

We claim:

1. A method of ameliorating or preventing, in a mammal, the symptoms of a severe inflammatory disorder that is associated with the production of an agent selected from the group consisting of endotoxin, protein-tyrosine kinase, cyclooxygenase-2, tumor necrosis factor alpha, interleukin-

1, interleukin-6, macrophage chemotactic protein, inducible nitric oxide synthetase, mitogen-activated protein kinase, macrophage inflammatory protein, interferon-gamma, tissue factor, granulocyte-macrophage-colony stimulating factor, and phosphotyrosine phosphatase; said method comprising administering to the mammal a therapeutically effective amount of an active sesquiterpene lactone, or a pharmaceutically acceptable salt of an active sesquiterpene lactone; wherein said active sesquiterpene lactone contains an α-methylene-γ-lactone functional group.

2. The method of claim 1, wherein the agent is selected from the group consisting of tumor necrosis factor alpha and interleukin-1.

3. The method of claim 1, wherein the agent is selected from the group consisting of cyclooxygenase-2 and mitogen-activated protein kinase.

4. The method of claim 1, wherein the severe inflammatory disorder is selected from the group consisting of sepsis and septic shock.

5. The method of claim 1, additionally comprising the step of administering to the mammal a bactericidal amount of an antibiotic.

6. The method of claim 1, wherein the active sesquiterpene lactone contains a functional group selected from the group consisting of an epoxide, a cyclopentenone, a cyclohexenone, a cyclohexadienone, an α,β-unsaturated ester, and an α, β-epoxy ester.

7. The method of claim 1, wherein the active sesquiterpene lactone contains a functional group selected from the group consisting of an epoxide, a cyclohexadienone, an α,β-unsaturated ester, and an α,β-epoxy ester.

8. The method of claim 1, wherein the active sesquiterpene lactone contains a cyclohexadienone functional group.

9. The method of claim 1, wherein the active sesquiterpene lactone is encelin.

10. The method of claim 1, wherein the active sesquiterpene lactone is parthenolide.

11. The method of claim 1, wherein the active sesquiterpene lactone is leucanthin B.

12. The method of claim 1, wherein the active sesquiterpene lactone is enhydrin.

13. The method of claim 1, wherein the active sesquiterpene lactone is melampodin A.

14. The method of claim 1, wherein the active sesquiterpene lactone is tenulin.

15. The method of claim 1, wherein the active sesquiterpene lactone is confertiflorin.

16. The method of claim 1, wherein the active sesquiterpene lactone is burrodin.

17. The method of claim 1, wherein the active sesquiterpene lactone is psilostachyin A.

18. The method of claim 1, wherein the active sesquiterpene lactone is costunolide.

19. The method of claim 1, wherein the active sesquiterpene lactone is cinerenin.

20. The method of claim 1, wherein said administering of the active sesquiterpene lactone is performed by subcutaneous injection, intravenous injection, or transdermal absorption.

21. The method of claim 1, wherein the mammal is a human.

22. A method of ameliorating or preventing, in a mammal, the symptoms of a post-reperfusion injury that is associated with the production of an agent selected from the group consisting of endotoxin, protein-tyrosine kinase, cyclooxygenase-2, tumor necrosis factor alpha, interleukin-1, interleukin-6, macrophage chemotactic protein, inducible nitric oxide synthetase, mitogen-activated protein kinase, macrophage inflammatory protein, interferon-gamma, tissue factor, granulocyte-macrophage-colony stimulating factor, and phosphotyrosine phosphatase; said method comprising administering to the mammal a therapeutically effective amount of an active sesquiterpene lactone, or a pharmaceutically acceptable salt of an active sesquiterpene lactone; wherein said active sesquiterpene lactone contains an α-methylene-γ-lactone functional group.

23. A method of ameliorating or preventing, in a mammal, the symptoms of a non-malignant or immunologically-related cell proliferative disease that is associated with the production of an agent selected from the group consisting of endotoxin, protein-tyrosine kinase, cyclooxygenase-2, tumor necrosis factor alpha, interleukin-1, interleukin-6, macrophage chemotactic protein, inducible nitric oxide synthetase, mitogen-activated protein kinase, macrophage inflammatory protein, interferon-gamma, tissue factor, granulocyte-macrophage-colony stimulating factor, and phosphotyrosine phosphatase; said method comprising administering to the mammal a therapeutically effective amount of an active sesquiterpene lactone, or a pharmaceutically acceptable salt of an active sesquiterpene lactone; wherein said active sesquiterpene lactone contains an α-methylene-γ-lactone functional group.

24. The method of claim 23, wherein the disease is selected from the group consisting of psoriasis, pemphigus vulgaris, Behcet's syndrome, acute respiratory distress syndrome, ischemic heart disease, post-dialysis syndrome, acquired immune deficiency syndrome, and lipid histiocytosis.

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